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VOLUME 40 NUMBER 5

ARCHIVES OF PATHOLOGY

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NOVEMBER-DECEMBER 1945

PUBLISHED MONTHLY BY AMERICAN MEDICAL ASSOCIATION, 535 NORTH DEARBORN STREET, CHICAGO 10, ILLINOIS. ANNUAL SUBSCRIPTION, $6.00

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of Congress of March 3, 1879

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NOTE

Owing to a strike of printers in the press of the American Medical Association, publication of all of the special periodicals of the Association has been considerably delayed. In order to bring these periodicals up to the normal schedule, the November and December numbers are combined into a single issue. Regular publication will be resumed with the issues for January 1946.

MANAGING EDITOR.

ARCHIVES OF PATHOLOGY

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EFFECTS OF VARYING DEGREES OF HYPOPHYSECTOMY IN THE DOG

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This investigation led directly from our failure to encounter certain of the classic hypophysectomy deficits in dogs in which great pains had been taken to perform hypophysectomies that would be total.[1] Yet the other classic deficits were encountered as expected.

Particular care had been taken to make certain that hypophysial remnants did not remain attached to the base of the brain. This was done by purposefully encroaching on the tuberal portion of the hypothalamus.[2] Equal precautions were taken to avoid leaving any glandular remnants in the base of the pituitary fossa, but in every instance a minute fragment of mixed hypophysial tissue was found in the scar tissue in the region where the artery of the posterior lobe penetrates the dura. (See arrow 2 in fig. 4 *E* for the location of and the relative size of such fragments.)

These animals exhibited (1) sexual regression, (2) maximal change in coat (it became soft and luxuriant), (3) obesity, (4) lowering of basal metabolism (30 per cent [1b]) and (5) permanent diabetes insipidus. Surprisingly, the carbohydrate metabolism and the adrenal function, as well as the size of the adrenal glands, were not noteworthily disturbed. There was no tendency toward hypoglycemia, either spontaneously or during starvation, and the animal's reaction to insulin was not strikingly altered from the normal (fig. 1). When the pancreas was removed, instead of a Houssay effect being obtained, there followed immediately hyperglycemia, which was more intense than that which follows pancreatectomy in an otherwise normal dog. In addition, death occurred as early as two to three days after the pancreatectomy. This was much earlier than death occurs normally following pancreatectomy (fig. 1). Thus the effect of removing the pancreas deviated from the usual, but in a direction opposite from that of the Houssay effect. No signs of hypoadrenalism were evident, and at autopsy it was observed that the adrenal cortex showed only moderate or no atrophy (dog 5Br, fig. 2).

The question arose, therefore, as to whether the failure of the expected deficits to appear in carbohydrate metabolism and in adrenal function and size was due to encroachment on the function of the hypothalamus or to potent selective activity on the part of the small remnant of hypophysial tissue remaining in the base of the fossa. These were the only two variables that deviated from an uncomplicated total hypophysectomy.

For an ultimate elucidation of this particular problem, it became highly desirable to have data available in terms of our own experience relative to the effects of varying degrees of hypophysectomy in the dog. This report comprises a more or less comprehensive summary of the results derived from such a study. From the content it will be appreciated that the information gained has not progressed in some aspects

From the Department of Physiology and Pharmacology, University of Alabama School of Medicine, and the Department of Physiology and Pharmacology, Baylor University College of Medicine.

This investigation was aided by grants from the Rockefeller Foundation to the University of Alabama and from the John and Mary R. Markle Foundation to Baylor University College of Medicine.

1. (*a*) Keller, A. D., and Bruhn, J. M.: Am. J. Physiol. **129**:396, 1940. (*b*) Keller, A. D.: ibid. **133**: 347, 1941.

2. Reichert, F. L., and Dandy, W. E.: Bull. Johns Hopkins Hosp. **58**:418, 1936.

beyond an orientation stage. Most of the pertinent points have been reported in preliminary form.[3]

OPERATIVE PROCEDURES

Operative Approach.—The subtemporal intracranial approach which was utilized by Paulesco and Cushing was used. The deviations from the Cushing procedure[4] were as follows: 1. Only one window was made in the bone. 2. The temporal muscle was cut across through its belly just caudal to and in line with the main surface artery supplying it, in addition to the cut Cushing made along its upper border of insertion. Such a T-shaped incision through this muscle facilitated the exposure of the underlying bone and permitted better visibility of the operative field. 3. The animals were anesthetized with pentobarbital sodium instead of with ether. The relaxation of the brain under this anesthetic is superb as compared with that under ether. 4. The head was stabilized in a Magnus head holder. This feature not only stabilized the operative field, thus aiding visibility, but also reduced obstruction of venous return to a minimum. Hypertonic solutions for shrinking the brain were never used.

The temporal lobe of the brain was retracted upward and inward with a broad, rigid brain spatula. By rotation of the head sidewise through appropriate manipulation of the head holder, the right border of the hypophysis, its stalk and the right surface of the hypothalamus were exposed to direct view. The retraction of the temporal lobe placed the hypophysial stalk on a slight stretch, and this also increased the visibility of these structures. In most instances, it was easy to place the jaws of the scissors about the stalk and cut it at any desired distance from its junction with the hypothalamus. Care must be taken not to extend the jaws of the scissors too far ventrally (to the left), since, if one does so, invariably the underlying internal carotid artery or a branch of this artery is cut. After the cutting of the stalk, the isolated gland was removed by appropriate manipulation with forceps or preferably with a small periosteal elevator that acted both as a spoon to lift the gland and as a knife to dislodge the posterior lobe attachment with the artery of the posterior lobe. When the stalk was cut proximal to the region of attachment of the posterior and anterior lobes (at or proximal to line 2, fig. 3), the entire isolated gland was usually removed as a complete surgical specimen without disturbing the relation of the posterior and anterior lobes in any way.[5]

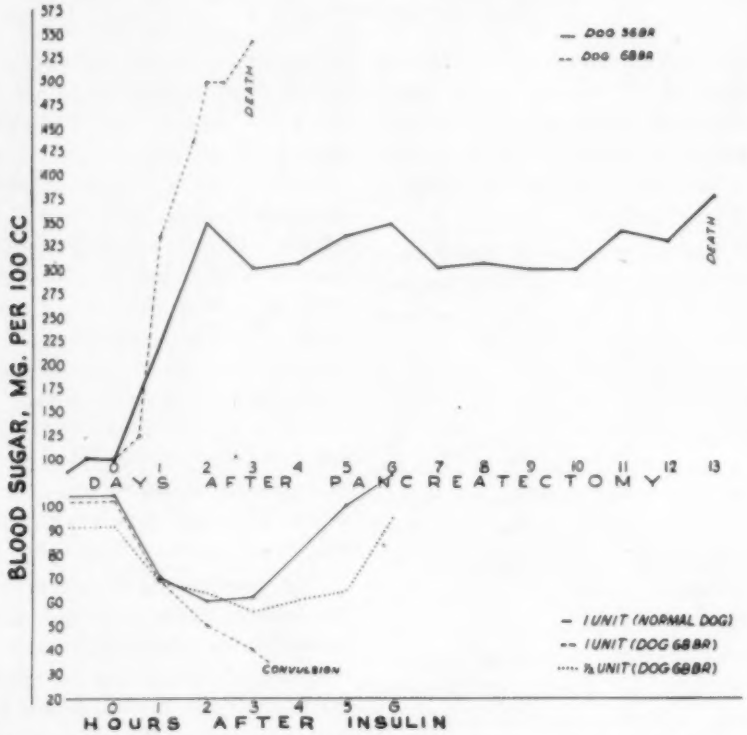

Fig. 1.—The blood sugar curve for dog 56Br is shown as representing a normal postpancreatectomy curve. This dog had approximately two thirds of the pars anterior removed several months before pancreatectomy.

The posthypophysectomy insulin tolerance and postpancreatectomy blood sugar curves for dog 68Br are shown to illustrate the failure of an appreciable lowering of the carbohydrate reserves in certain of our dogs in which, in addition to total hypophysectomy, there was macroscopically detectable encroachment on the hypothalamus (reversal effect). See text.

3. (*a*) Keller, A. D.; Bruhn, J. M.; Barton, C. F., and Lawrence, W. E.: Federation Proc. **1**:46, 1942. (*b*) Bruhn, J. M.; Keller, A. D.; Lawrence, W. E., and Barton, C. F.: ibid. **1**:11, 1942. (*c*) Keller, A. D.: ibid. **3**:23, 1944; (*d*) **3**:23, 1944.

4. Crowe, S. J.; Cushing, H., and Homans, J.: Bull. Johns Hopkins Hosp. **21**:127, 1910.

5. Keller, A. D., and D'Amour, M. C.: Arch. Path. **21**:185, 1936 (fig. 5, p. 195).

When the hypophysis was exposed by this approach, the demarcation of the various anatomic structures was remarkably clear and definite. The tissue of the stalk had a characteristic semitranslucent appearance that contrasted sharply with the whitish appearance of the tissue of the hypothalamus and the whitish tissue of the pars anterior. If one dissects a fresh surgical specimen of the hypophysis in which the stalk has been severed at its connection with the hypothalamus, the juncture of the tissue of the pars tuberalis with that of the pars anterior is readily distinguishable. Frequently in the dog, and essentially invariably in the cat, the pars tuberalis can be pulled free from the pars anterior by gentle traction. Accordingly, at operation one can estimate the level of section of the stalk and the extent of removal of the gland by direct visibility and particularly by inspection of the surgical specimen.

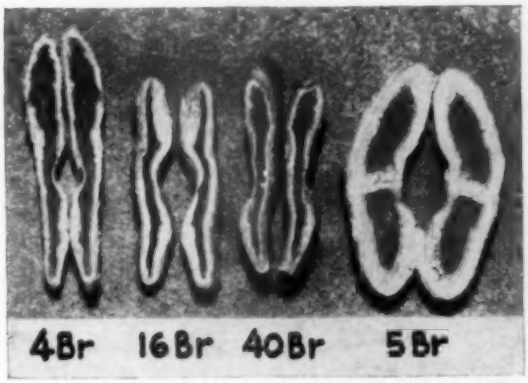

Fig. 2.—Photograph of left adrenal glands from dogs having different types of hypophysectomy.

Dog 4Br was a near-total hypophysectomized preparation which was maintained five months after the operation. Its tolerance for insulin was decreased in the neighborhood of sixty times. The pancreas was not removed. Note that the cortex is thinned but that the thinning is not so great as that in the adrenal gland of dog 40Br.

Dog 40Br was an animal with an anatomically uncomplicated total hypophysectomy which died in a crisis suspected as being due to adrenal insufficiency four weeks after operation. Insulin tolerance tests were not run on this animal. Note that the cortex is reduced to paper-thin thickness; there is definitely greater thinning in this adrenal gland than in those of dogs 4Br and 16Br.

Dog 16Br underwent total hypophysectomy with mild antomic encroachment on the hypothalamus. This animal was maintained six months after operation. Its insulin tolerance was decreased in the neighborhood of sixty times. Pancreatectomy resulted in a typical hypophysectomy-pancreatectomy crisis terminating in death in five hours. This dog therefore exhibited a mild reversal effect with respect to both adrenal size and carbohydrate metabolism. Note that the thinning of the cortex in this adrenal gland approximates that in the adrenal gland of dog 4Br.

Dog 5Br underwent total hypophysectomy plus drastic anatomic encroachment on the hypothalamus. This dog was maintained for one year after operation. Insulin tolerance was decreased in the neighborhood of ten times. The pancreas was not removed. Note that the adrenal size, if it deviates from the normal, is toward hypertrophy (marked reversal effect).

Following removal of the surgical specimen, the cut surface of the stalk is directly visible, and the amount of tuberalis tissue remaining behind can usually be estimated. In the event that bits of pars anterior remain, they stand out prominently as whitish specks or nodules.

Devitalization of Possible Remnants of Hypophysis.—Early in the series, after the removal of the surgical specimen, the base of the fossa was pressure swabbed with a small pledget of cotton and later cauterized lightly in an attempt to remove, or at least to devitalize, all glandular remnants. This procedure proved to be inadequate, since cautery frequently opened the adjoining venous sinuses. Even when cautery was successful without bleeding of sinuses, the incidence of failure to devitalize the fragment of glandular tissue associated with the artery of the posterior lobe proved to be too frequent. Later, we swabbed the fossa with a pledget of cotton soaked in concentrated phenol. This also was found to open the venous sinuses frequently, and its penetrative power was not sufficient to involve routinely the remnants of tissue frequently located within the dural canal of the artery. We have subsequently found what would seem to be the ideal procedure for this purpose, namely, pressure swabbing of the base of the fossa with a pledget of cotton soaked in solution of formaldehyde U. S. P. This has proved to be effective in devitalizing all glandular remnants without great danger of precipitating undesirable bleeding. This procedure was used in most of the experiments reported in the succeeding pages.

Sealing the Ventricular System.—Finally, before closure, in the instances in which the stalk was cut near the hypothalamus in such a way that the third ventricle was opened (lines 2 to 5, fig. 3) a small bit of temporal muscle was placed adjacent to the cut surface of the stalk to insure the immediate sealing of the opened ventricular system.

CARE OF ANIMALS

Immediately after operation, the animals were placed in an incubator held at 29 C. until shivering was prominent. They were then placed in ordinary cages located in a room maintained at 24 to 26 C.

The animals were not fed until the morning after operation, at which time a multiple feeding regimen was instituted. They were fed solid food every eight or twelve hours, and sugar was placed in the drinking water. The daily meal of 50 Gm. of wet Baloration or Purina meal per kilogram of body weight was given one-third or one-half at a time.[6] In the later part of the series, the total intake of food was limited to a maximum of 300 Gm. daily, regardless of the size of the dog. Thus the total intake of food was not increased if and when obesity developed. This diet was continued until the status of the animal's carbohydrate reserves was determined. With those in which the reserves were low, the multifeeding regimen was continued, but only with extremely sensitive animals was the addition of sugar to the drinking water continued. Those animals

6. Baloration is the dog meal marketed previous to the war by Tioga Mills, Waverly, N. Y. Purina meal is the kennel dog chow meal marketed by Ralston Purina Company, St. Louis. This meal is stated by the manufacturers to consist of meatmeal, dried skim milk, a vitamin G concentrate, carotene, cod liver oil, brewers' dried yeast, wheat germ, corn grits, wheat cereal, corn cereal, dried beet pulp, molasses, steamed bone meal and iodized salt.

in which the reserves remained normal or near normal were returned to the usual regimen of one feeding per day. All animals were fed by force if they did not eat of their own volition.

ASSAY OF FUNCTIONAL DEFICITS

Carbohydrate Reserves. — The status of the animal's carbohydrate reserves was determined (1) by observing the postabsorptive blood sugar level, (2) by determining the blood sugar level curve after various periods of fasting and (3) by making insulin tolerance tests. We prefer to use the term "tolerance" rather than "sensitivity" in reference to the insulin test because the animal's ability to tolerate a given dose of insulin is measured over the entire period that the insulin is acting. rate and to the same level during the first hour or hour and a half. Following this period, if the dose of 1/40 unit has been used, the animal will go into a fatal hypoglycemic crisis if sugar is not administered, but if 1/80 unit has been given it will successfully combat this dose without sugar.

In assaying the status of the carbohydrate reserves of the animal with the insulin tolerance test, we determined the largest dose of insulin per kilogram of body weight which the animal could tolerate with spontaneous recovery, and the smallest dose of insulin per kilogram of body weight that would precipitate a crisis requiring sugar therapy for recovery. A major convulsion or more or less continuous minor convulsions were the criteria used as indicating the need for sugar therapy.

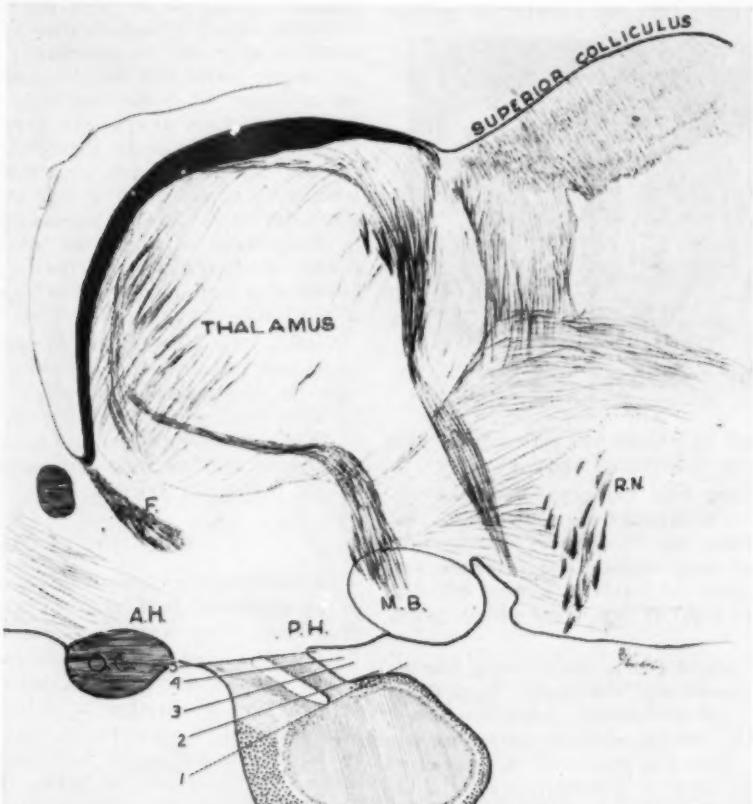

Fig. 3.—Semischematic drawing of a sagittal section taken through the diencephalon and the hypophysis, on which the different types of hypophysectomy are indicated by lines 1 to 5. Removal of the tissue distal to line 1 constitutes a near-ordinary hypophysectomy; that to line 2, an ordinary hypophysectomy; that to line 3, an ordinary-plus hypophysectomy; that to line 4, a near-total hypophysectomy, and removal to line 5, a total hypophysectomy.

O.C. is the optic chiasm; *A.H.*, the anterior hypothalamus; *P.H.*, the posterior hypothalamus; *F.*, the fornix; *M.B.*, the mamillary body, and *R.N.*, the red nucleus.

We have learned that if attention is given only to the rate of fall of the blood sugar for a limited period after the injection of insulin the data obtained are misleading in certain respects. For instance, the tolerance for insulin is markedly reduced from the normal in dogs which have undergone near-total hypophysectomy. In such a dog, whether a dose of 1/40 unit of insulin per kilogram of body weight or a dose of 1/80 unit has been given, the blood sugar will usually drop at the same

The blood sugar reading has not been taken as the primary criterion in the insulin tolerance tests. We have used the symptomatic complex in the animal for this purpose. It is necessary to determine the blood sugar, however, in order to be certain of uniform absorption of insulin from the subcutaneous depot. The insulin was administered beneath the skin of the dorsum of the neck. This region is uniformly free of fat even in a maximally obese preparation.

In this particular series of animals, the insulin tolerance tests were not begun earlier than two months after hypophysectomy, and succeeding tests were separated by at least two weeks. This procedure safely avoids the possibility of the effects of previous doses of insulin on subsequent tests. In most instances, the animals were maintained for six months after hypophysectomy, and the level of the animal's insulin tolerance was always verified just previous to pancreatectomy or the termination of the experiment. In our experience the animal's insulin tolerance remains permanently constant after a posthypophysectomy period of two months.

Function After Pancreatectomy.—In from three to six months after hypophysectomy the animals were pancreatectomized, care being taken to make certain that the operation was total. Following this operation, the blood sugar level was determined, and the usual urinary studies were made daily for the purpose of determining the presence or the absence of diabetes mellitus.

In the event that a Houssay effect developed, its magnitude was assayed by performing dextrose tolerance tests, in which 2 Gm. of dextrose per kilogram was administered by stomach tube (fig. 7). Although originally the animals showing a Houssay effect were allowed to live until death occurred, their lives are now terminated after a given postpancreatectomy interval. In this way another criterion for evaluating the magnitude of the Houssay effect manifests itself, namely, the amount of fat in the liver as noted grossly, histologically and chemically.

When a Houssay effect was obtained, careful inspection was made at autopsy for gross remnants of pancreatic tissue. Further, the fatty tissue attached to the duodenum where the pancreas had been attached was removed, sectioned serially and inspected carefully histologically for evidence of pancreatic remnants.

We did not in any instance attempt to modify the effects of the pancreatectomy by administering insulin, lipocaic or choline or by feeding fresh or desiccated pancreas.

Adrenal Function.—Early in the series, animals in which it was believed probable that hypofunction of the adrenal glands would result were watched closely for evidence of spontaneous crises of adrenal insufficiency. Later, in addition to this procedure, salt was abruptly withheld from the diet to see if a crisis could be precipitated. In both instances, care was taken to prevent hypoglycemia from complicating the picture. When crises were precipitated, adrenal cortex extract (Upjohn) and desoxycorticosterone were used to insure recovery and maintenance.

Sexual Activity and External Genitalia.—The external genitalia were inspected at regular intervals for change. Evidence of estrual activity, as well as suggestive regression in size, was noted. In the instances in which estrual phenomena appeared, the dogs were placed in association with males known to be active and potent. If copulation occurred, the female was maintained at least nine weeks to determine whether pregnancy resulted.

Coat.—The animal's hair was described at the time of operation and redescribed two or three months after hypophysectomy. There is no question when the texture of the coat is fully changed. The coarse, adult type of hair is replaced by soft, luxuriant hair which is definitely infantile in type. Partial changes in coat are often readily evident, but frequently they are difficult to evaluate with certainty.

Growth.—Growth has been studied in 1 puppy and also in a fairly sizable number of "senior pups." The only end point used was that of noting whether they maintained the stature present at the time of operation as well as their puppy appearance. Evaluation of stature and growth curves is difficult because of the onset of obesity; accordingly our observations on growth can be summarized only on the basis of impressions. The factor of growth will be studied in a subsequent investigation. Roentgen technics, as well as litters of puppies of known strains, will be used, and appropriate normal puppies will be maintained as control material.

Obesity.—Obesity was evaluated by general appearance, weight curves and the amount of fat found in the abdominal cavity by inspection at autopsy, as well as the measured depth of the subcutaneous layer over the dorsum of the hips. These criteria were rendered more reliable by maintaining the animals on a constant daily intake of food which had previously been rigidly determined to be adequate yet not excessive (fat-producing) in normal dogs under the same cage regimens over the same periods (six to twelve months).

Energy Metabolism.—At the suggestion of one of us (A. D. K.), Dr. J. M. Bruhn took advantage of the opportunity of studying the status of the energy metabolism in many of the animals of this series. It is presumed he will make a separate complete report on this phase of the work. He has summarized the metabolic data obtained in combined preliminary reports.[7]

POSTMORTEM STUDIES

Hypophysis-Hypothalamus.—When the animals died or their lives were terminated, careful autopsies were made. The brain was removed with the fossal scar and dural tissues remaining undisturbed and still attached to the ventral part of the hypothalamus. This procedure is accomplished with ease in the dog. After fixation in solution of formaldehyde U. S. P. (1:10) in the early portion of the series, and Zenker's solution prepared with solution of formaldehyde in place of glacial acetic acid in the later portion of the series, the hypophysis-hypothalamus blocks were sectioned sagittally and serially. Appropriate sections were mounted and stained with a modified Maximow procedure. The criterion used for delimiting the pars anterior was the presence of acidophils.

Adrenal Glands.—The size and the appearance of the adrenal glands were noted grossly. The thickness of the cortex was measured after the gland had been cut longitudinally through its center with a razor blade. The latter procedure was carried out after appropriate fixation (fig. 2). There was a grayish or a blackish surface tinge rather than the normal white color in the unfixed adrenal gland in which the cortex was markedly atrophied. This change in surface color was due to the dark tissue of the medulla being visible through the thinned cortex. (Look carefully at the photograph of the adrenal gland of dog 40Br, figure 2.) The gland is also distinctly flattened as compared with the rounded normal adrenal gland. (Note the definite flatness of the glands from dogs 4Br, 16Br and 40Br as compared with that of dog 5Br in figure 2.) These changes are striking when maximal or near-maximal in intensity. Over-all size and weight vary so markedly in female dogs that it is difficult to evaluate intermediate stages of atrophy. It is hoped that subsequent histologic analyses will be helpful in this respect.

Liver.—The liver was inspected carefully at the time the abdomen was opened for removing the pan-

7. Keller and Bruhn.[1a] Bruhn and others.[3b]

creas. At autopsy the liver was again inspected carefully, particularly for color. In order to make the observations on color more reliable, the blood was washed out with saline solution by perfusion through the left side of the heart. A fatty liver appears intensely yellow when it is washed free of blood. By using color in this way, it is believed intermediate grades of fatty infiltration can be estimated by plus signs as reliably as the amount of sugar in a qualitative sugar urinalysis.

COMMENT ON OPERATIVE PROCEDURES AND RESULTS

We have found that the following described and illustrated types of hypophysectomy can be

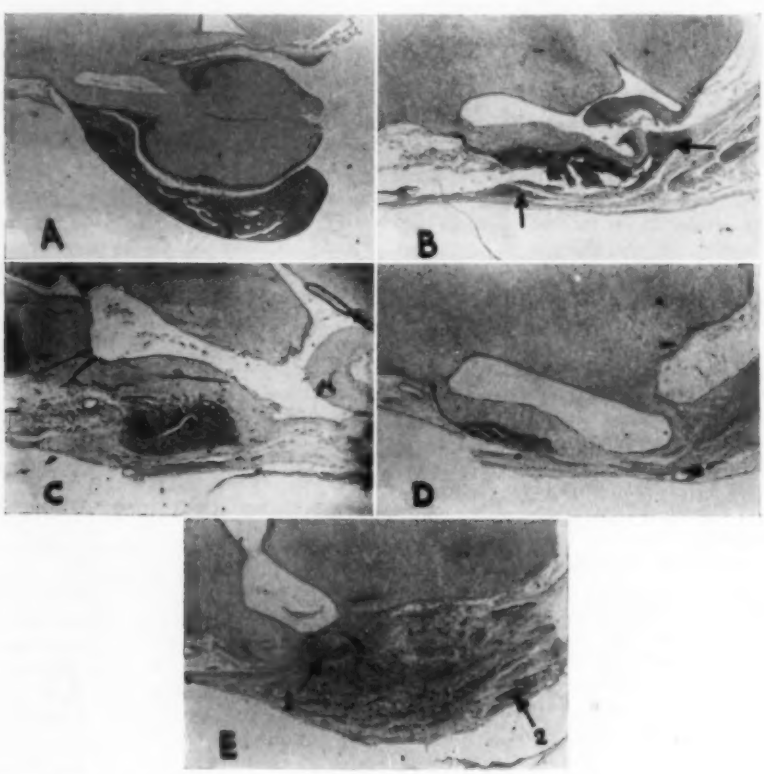

Fig. 4.—Photographs of sections taken from the series of dogs having varying degrees of hypophysectomy. All sections were stained by a modified Maximow method.

A, sagittal section through a normal dog's hypophysis, showing the relationship of the various components of the hypophysis and the attachment of this structure to the hypothalamus. Note that by careful inspection the pars tuberalis is distinguishable from the pars anterior and the pars intermedia. The presence of the acidophils in these two components gives them a more intense shade than the pars tuberalis. Note that the pars intermedia surrounds only the infundibular process, there being no indication of its extending upward about the infundibulum (stalk). Compare these demarcated features with those indicated in the schemata in figure 3.

B, sagittal section taken from the series on dog 137Br, illustrating a near-ordinary hypophysectomy. Note (1) that the pars tuberalis is largely intact, including the narrow strap of tissue extending cephalad along the very proximal part of the infundibulum, (2) that the infundibulum is not disturbed (there is no indication of shrinking or sloughing in this structure); (3) that the third ventricle is not distorted in any way, (4) that readily recognizable remnants of pars anterior remain encircling the distal end of the stalk. These remnants are indicated by the arrows. Compare this section with line 1 in figure 3.

C, sagittal section taken from the series on dog 217Br, illustrating an ordinary hypophysectomy. There is no detectable evidence of any remaining pars anterior, with considerable pars tuberalis intact. Pars tuberalis is also present caudally out of range of the field photographed. This section is a duplicate or near-duplicate of the sections illustrated by S. Koster and A. Geesink (Arch. f. d. ges. Physiol. **222**:293, 1929) in which, they asserted there was evidence of postoperative hyperplasia of the pars tuberalis tissue. Compare with line 2 in figure 3.

D, sagittal section from the series on dog 171Br, illustrating an ordinary-plus hypophysectomy. Note that much of the pars tuberalis and of the infundibulum has been removed but that the proximal portions of these structures remain unaltered. Compare with line 3 in figure 3.

E, sagittal section taken from the series on dog 134 Br, illustrating a near-total hypophysectomy. Note that all but the very proximal tip of the infundibulum has been removed and that only a small island of pars tuberalis remains (quarter-moon-shaped tissue at end of arrow 1). The small nodule of tissue at the end of arrow 2 is a glandular remnant which remained in the fossal scar tissue. Acidophils were recognized in this remnant. The rest of the tissue seen in the photograph is organized muscle which was placed in the fossa at the time of operation. Compare this section with line 4 in figure 3.

executed, with certain limitations, at will. The deficits that result are reliably predictable on the basis of the criteria outlined in the foregoing pages. The total number of dogs which were maintained sufficiently long after a given type of hypophysectomy to obtain observations pertinent to the investigation was in the neighborhood of 300. In these the experiments were not all carried through to pancreatectomy. The work has engaged our divided attention since the summer of 1939.

NEAR-ORDINARY HYPOPHYSECTOMY

In the near-ordinary type of hypophysectomy, the surgical specimen included all of the posterior lobe (the infundibular process and the pars intermedia), a fragment of the pars tuberalis and roughly more than 90 per cent of the pars anterior (line 1 in fig. 3 and section from dog 137Br in fig. 4*B*). The tissue which remained behind undisturbed—attached to the hypothalamus—consisted of most of the infundibulum, the larger portion of the pars tuberalis and various-sized fragments of the pars anterior. In executing this type of hypophysectomy, the stalk was cut through at its distal extent so that a visible remnant of the proximal portion of the pars anterior was seen remaining behind encircling the free cut end of the stalk. Although it is suspected that the tissue immediately adjacent to the cut surface always deteriorated, a goodly portion of the remaining pars anterior usually continued viable and was readily identified by examination of serial histologic sections and frequently by inspection of the gross specimen at autopsy.

Carbohydrate Reserves.—These preparations exhibited a decrease in tolerance for insulin in the neighborhood of five times. The manner in which insulin tolerance was affected by the surgical manipulation involved in removing varying amounts of the pars anterior is illustrated by dogs 56Br, 81Br and 137Br.

In dog 56Br the uppermost two thirds (right) of the pars anterior was removed selectively without disturbing the underlying one third (left) or the tissues of the posterior lobe and stalk. These facts were verified by serial histologic cross sections. This dog was unable to tolerate 1 unit of insulin per kilogram of body weight but did tolerate successfully ½ unit. Normal dogs never failed to tolerate successfully 1 unit per kilogram. (See curve for normal dog, figure 1.)

In dog 81Br, at operation, the globular gland was cut across through what was judged to be near the middle of the anterior and posterior lobes. Histologic serial sections demonstrated, however, that approximately one tenth or less of the proximal portion of the pars anterior remained, whereas the whole of the posterior lobe was absent. The tissues of the stalk were undisturbed. This dog tolerated ¼ unit of insulin per kilogram of body weight in normal fashion but could not successfully combat ½ unit.

The same operative procedure was carried out on dog 137Br as on dog 81Br except that the gland was cut through near the proximal extent of the pars anterior in such a way that only a narrow ring of this tissue remained attached to the stalk. (See section for dog 137Br in figure 4*B*.) Histologically, there was an easily recognizable remnant of pars anterior remaining in good condition, but it was less than that present in dog 81Br. This dog succeeded in combating successfully, although with some difficulty, ¼ unit of insulin per kilogram of body weight. The hypoglycemic symptoms were profound.

The near-ordinary hypophysectomized dogs in the chronic state never exhibited any tendency toward spontaneous hypoglycemia, nor did their blood sugar levels deviate from the normal as a result of periods of fasting. Most of these dogs, in which the stalk was cut through its extreme distal portion, did exhibit a much increased susceptibility to spontaneous hypoglycemia during the days immediately following operation.

Function After Pancreatectomy.—Following pancreatectomy, the near-ordinary hypophysectomized dogs invariably exhibited maximal and typical permanent diabetes mellitus. Their livers became uniformly markedly fatty.

Adrenal Function and Size.—These dogs in the chronic state never exhibited any tendency toward crises of adrenal insufficiency. Their ability to withstand a lowered amount of, or an absence of, sodium in, the diet did not deviate outwardly from that of the normal dog. At autopsy the adrenal glands always appeared within the normal range in size, color, roundness and thickness of cortex.

Sexual Activity and External Genitalia.—The response of sexual functions to this type of operation was variable. Usually all sexual activity was completely eliminated, with failure of infantile organs to develop and regression of adult organs, depending on the status of these organs at the time of the hypophysectomy. Nevertheless, the retention of sexual activity to a limited degree and the failure of the sexual apparatus to regress were in evidence surprisingly frequently even when only a relatively small amount of recognizable pars anterior tissue remained attached to the stalk. The external genitalia exhibited no tendency toward regression in size. Periodically they became enlarged, and bleeding from the vaginal orifice occurred. Outwardly these signs of estrus did not deviate from the normal.

In 1 instance (dog 34Br), during the estrual periods the animal received the male. Successful copulation occurred several times during each estrual period, but pregnancy never resulted. A most interesting feature in this animal was that several typical periods of estrus occurred which were spaced only a month apart rather than the usual six months apart.

In several instances, external signs of estrus appeared in animals exhibiting a sufficiently striking change in coat for it to be judged maximal in extent.

Growth.—Our impression with respect to growth has been that complete cessation of growth has occurred in all senior pups on which near-ordinary hypophysectomy was done. In 1 instance in which the procedure was executed in a less than half-grown puppy, dwarfing definitely resulted (dog 172Br). In this animal there was a maximal change in coat; there was complete failure of sexual development; the insulin tolerance was decreased only in the range of five times; typical diabetes mellitus resulted from removing the pancreas; obesity was in evidence, and accordingly dwarfing was not detectable from observing the weight curve; at autopsy the adrenal glands were grossly within the normal range.

Obesity.—Definite obesity was in evidence in the preparations of this type which we maintained over a period of a year. Several preparations which were maintained for only three to five months had not shown what was estimated as definite obesity. The weight curve for dog 81Br demonstrates the presence of

definite obesity, which developed relatively rapidly. This suggested it would have become marked had the animal been maintained longer (fig. 9).

ORDINARY[8] HYPOPHYSECTOMY

In the ordinary type of hypophysectomy, there remained intact as much of the tissue of the stalk as was compatible with total removal of the pars anterior (line 2 in fig. 3 and section *C* from dog 217Br in fig. 4). This end result was attained at operation in two ways: First, when the stalk was sectioned in such a way that only a small rim of pars anterior tissue remained attached to the stalk, this tissue frequently did not survive the operation, as evidenced by histologic inspection. In dog 184 Br one has an example of an ordinary hypophysectomy attained in this way. Second, an ordinary hypophysectomy was done with certainty with high incidence by placing the blades of the scissors so that the stalk was cut just at the cephalic border of the pars anterior. In these instances the gross inspection of the surgical specimen showed an uncut pars anterior with a minimal amount of pars tuberalis attached to it. In dog 217Br there is an example of an ordinary hypophysectomy attained in this way.

Marked Transitory Increased Susceptibility to Hypoglycemic Crises.—While the latter of the two procedures mentioned in the foregoing paragraph has been found to be readily executed surgically, it was difficult to maintain these animals to chronicity, definitely more so than it was to maintain the near-total hypophysectomized preparations. They recovered from the operation uneventfully but were lost in from a few days to three weeks after operation in crises in which hypoglycemia was a prominent feature. On one occasion we concentrated on doing only this type of operation for several months. A sizable number of animals was involved. Great care was taken to fortify the carbohydrate supply by frequent feeding and to make frequent periodic observations.

In spite of these precautions, we succeeded in maintaining only 3 of these preparations to chronicity. In dog 217Br is an example of an animal prepared in this way; it is one of the 3 mentioned. (See section *C* in figure 4.) In the chronic state there was no tendency toward hypoglycemia. It is obvious that the increased susceptibility to spontaneous hypoglycemia that was encountered in the preparations in which near-ordinary hypophysectomy was attempted was due to the same underlying cause as in these preparations.

Carbohydrate Reserves.—These dogs as a rule exhibited in the chronic state a slightly greater decrease of tolerance for insulin than did the near-ordinary hypophysectomized animals, ranging in the neighborhood of ten times. This was not invariable, however. For instance, dog 184Br tolerated 1/10 unit of insulin per kilogram of body weight and did not succeed in combating 1/5 unit (fig. 5), whereas dog 217Br combated successfully 1/5 unit but could not tolerate ½ unit.

Function After Pancreatectomy.—Typical diabetes mellitus, which seemed to be full blown, followed removal of the pancreas. Dog 184Br is representative (fig. 5). In this animal, as in the others, a maximal change in coat was in evidence.

Adrenal Function and Size.—These preparations never exhibited any signs of adrenal insufficiency after the chronic state was attained, either under normal feeding conditions or under the stress of a lowered level of sodium in the diet. They tolerated pancreatectomy in an entirely normal manner. At autopsy, although mild atrophy was suspected, grossly the adrenal glands could have been within the normal range for over-all size, color, roundness and thickness of cortex.

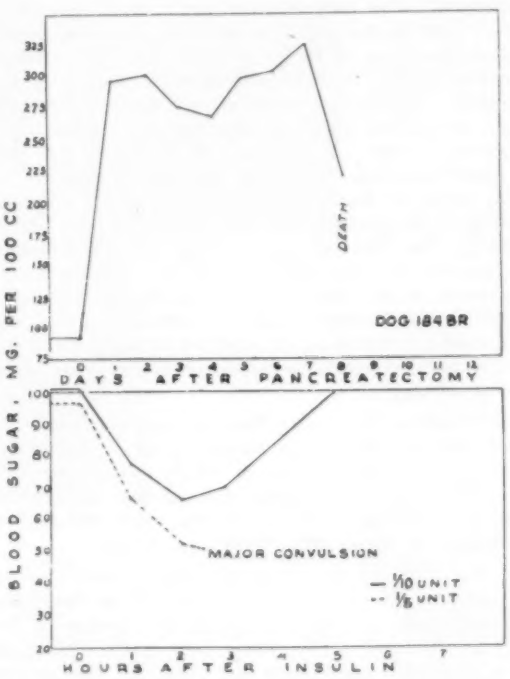

Fig. 5.—Blood sugar curve of dog 184Br (an ordinary hypophysectomized preparation), demonstrating a decrease of insulin tolerance of only ten times and the usual postpancreatectomy hyperglycemia with death in eight days.

Sexual Activity and External Genitalia.—Complete cessation of sexual activity and failure of development of, or signs of regression of, the sexual organs were invariably encountered in these preparations.

Growth.—We are certain that there was complete cessation of growth in the "senior" pups in which this type of operation obtained. Invariably a maximal change in coat from the adult to the infantile type resulted from the operation.

ORDINARY-PLUS HYPOPHYSECTOMY

In the ordinary-plus type of hypophysectomy, all the tissues of the posterior lobe and the pars anterior and the distal portion of the pars tuberalis and infundibulum were removed. The proximal portion of the pars

8. The use of the word "ordinary" is perhaps a poor choice because, judging from the functional results obtained, this type of hypophysectomy is not the one that has been ordinarily executed in the hands of most investigators. The term does fit, however, in the sense that it is universally assumed that the deficits precipitated by hypophysectomy as it is ordinarily executed are due to removal of the pars anterior per se. It is in this sense that the term "ordinary" is used in this instance; e. g., it is used as indicating the complete removal of the pars anterior.

tuberalis and infundibulum remained undisturbed (line *3* in fig 3 and section from dog 171Br in fig. *D*)

This type of preparation was not obtained at will. Dog 171Br and dog 58Br were such animals; dog 171Br resulted from an attempt to prepare a near-ordinary hypophysectomy, and dog 58Br resulted from an attempt to prepare an ordinary hypophysectomy.

Carbohydrate Reserves.—These preparations exhibited a varied reduction in tolerance for insulin. Dog 58Br tolerated 1/15 unit of insulin per kilogram of body weight but did not succeed in combating 1/10 unit. On the other hand, dog 171Br tolerated 1/10 unit but did not survive ¼ unit without sugar therapy (fig. 6). Dog 54Br tolerated ⅛ unit, but ⅓ unit precipitated a major convulsion which required sugar therapy. Although in most instances the animal's tolerance for insulin was reduced more than in the two previously described categories, never was the decrease so striking as in the near-total preparations described in the following section.

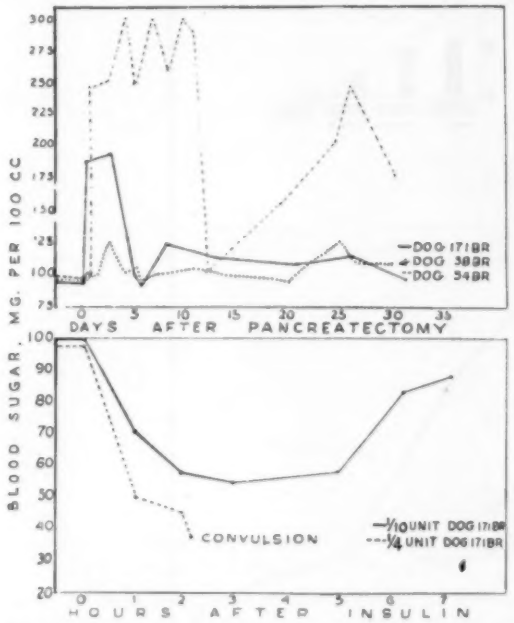

Fig. 6.—Blood sugar curves of dog 171Br (an ordinary-plus hypophysectomized animal), showing a post-hypophysectomy decrease in insulin tolerance of ten times and a normal postabsorptive blood sugar level following pancreatectomy (near-maximal Houssay effect).

The postpancreatectomy postabsorptive blood sugar curves for dogs 58Br and 54Br are also shown. Dog 58Br's curve shows no deviation from the normal (maximal Houssay effect). It was necessary to withhold food from dog 54Br for seventy-two hours to bring it down to the normal range (mild Houssay effect).

These animals in the chronic state did not exhibit any tendency to hypoglycemia, either spontaneous or precipitated by short periods of fasting.

Function After Pancreatectomy.—It was in these preparations that we encountered the Houssay effect as illustrated by the data for dogs 58Br, 171Br, 54Br and 186Br in figures 6 and 7. Our present data show a definite correlation between the occurrence and magnitude of the Houssay effect and the amount of stalk tissue remaining behind. The pars tuberalis is suspected of being the crucial tissue rather than the infundibulum; nevertheless, neither has been ruled out. Roughly one half of the stalk tissue must be removed before the Houssay effect appears, and approximately two thirds of this tissue must be removed before the effect is near-maximal.

The maximal amelioration of pancreatic diabetes obtained by us is illustrated in dog 58Br. In this dog there were complete absence of glycosuria, acetonuria and a normal or below-normal postabsorptive blood sugar level. The only deviation from the normal was a slightly raised dextrose tolerance curve.

A mild Houssay effect is evidenced by absence of acetonuria and presence of marked glycosuria, post-absorptive hyperglycemia and a markedly raised dextrose tolerance curve (dogs 54Br and 186Br, fig. 7). In such preparations it is necessary to withhold food for two or three days to reduce the blood sugar to normal or near-normal preparatory to running a dextrose tolerance test. This procedure itself raises the tolerance curve, but in the Houssay animal the curve is raised entirely out of proportion to the starvation effect. If such animals are fed daily, the only manifestations of the Houssay effect are the absence of acetonuria and the prolongation of the survival time following pancreatectomy. These features are illustrated in dog 54Br (figs. 6 and 7).

Adrenal Function and Size.—While we did not encounter any evidence of hypofunction of the adrenal glands in these animals, they tolerated pancreatectomy successfully, and the adrenal glands at autopsy exhibited variable evidence of atrophy of the cortex, which was judged to range from mild to moderate in extent.

Growth.—Our observations on growth in these preparations do not deviate in any way from those on growth in ordinary hypophysectomized puppies.

NEAR-TOTAL HYPOPHYSECTOMY

In these animals, all of the hypophysial tissue save the very proximal portion of the pars tuberalis and infundibulum was removed (line *4* in fig. 3 and section from dog 134Br in fig. 4 *E*). The pars tuberalis and infundibulum remaining were mere fragments as compared with the total original tissue. The hypothalamus was never encroached on, nor was the third ventricle distorted in any way, as evidenced by serial histologic studies. The presence of glandular fragments in the fossal scar tissue never altered the physiologic status of these preparations so far as could be determined. (See section for dog 134Br in figure 4 *E*).

Carbohydrate Reserves.—Near-total hypophysectomized animals recovered from the operation uneventfully, but hypoglycemic crises were prone to be precipitated; nevertheless, it was not difficult to maintain most of these animals indefinitely by care in appropriate feeding. Most of the preparations in the chronic state had normal blood sugar levels, taken eight or twelve hours after feeding. A few, however, had consistently low blood sugar levels in spite of the frequent feeding regimen. There was no observable correlation between this persistent low level of blood sugar and the animal's tolerance for insulin. For instance, dog 140Br could not tolerate 1/200 unit of insulin per kilogram of body weight when tested eight hours after taking food, yet her blood sugar level was within the normal range. On the other hand, dog 91Br had a persistent low level of blood sugar but was able to tolerate success-

fully 1/60 unit of insulin, although she could not tolerate 1/40 unit. We are at present unable to explain these low blood sugar levels.

Insulin tests were run on these animals either eight or twelve hours after the previous meal, rather than twenty-four hours after feeding as in the preparations described previously. The tolerance for insulin was invariably decreased markedly, the decrease being usually in the range of sixty times (dog 134Br, fig. 8). In a few instances, however, the decreases were as great as one hundred and two hundred times. In estimating the change in insulin tolerance, the decrease is actually greater in this type of preparation than the figures listed indicate, since the tests were run only twelve or even eight hours rather than the standard twenty-four hours after feeding.

onset and progress of the crises noted. The animals recovered from the pancreatectomies uneventfully and appeared entirely normal for several hours. Then, depending on the individual preparation, in from three to twelve hours a characteristic syndrome appeared. The animals became weak, lost interest in their environment and finally, when they were unable to stand, periodic straining movements set in, respiration became rapid, and death occurred after a characteristic period of gasping.

Adrenal Function and Size.—These animals invariably exhibited extreme atrophy of the adrenal glands. The organs were much flattened; they had lost their roundness; they appeared dark grayish or blackish according to the degree of thinning of the cortex. This was due to the fact that the underlying blackish medulla could

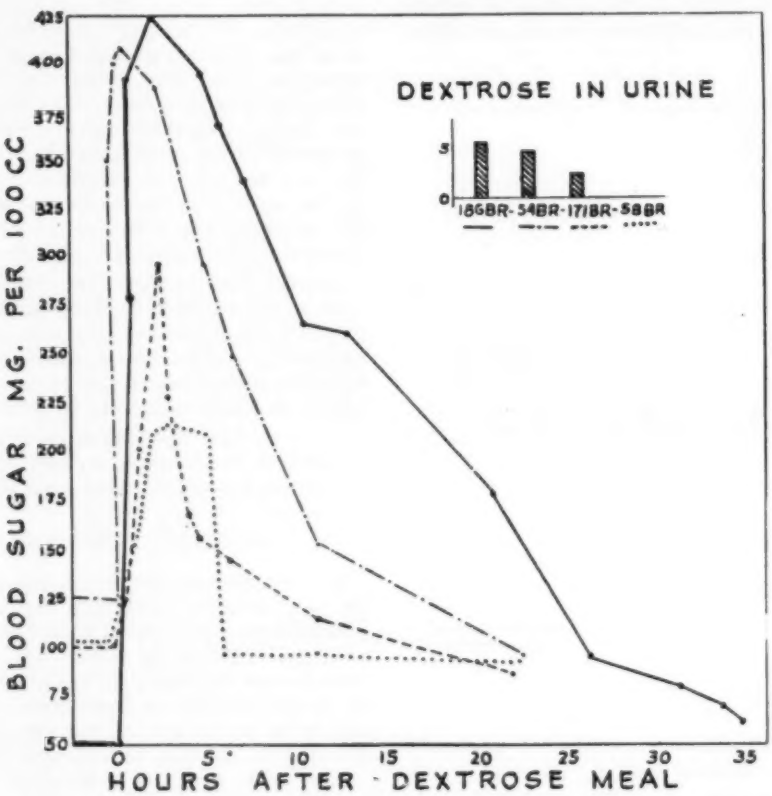

Fig. 7.—Dextrose tolerance curves for dogs 58Br, 171Br, 54Br. and 186Br, showing the efficacy of this method in assaying the magnitude of a given Houssay effect. The dextrose meal was 2 Gm. per kilogram, given by stomach tube.

Function After Pancreatectomy.—In these animals removal of the pancreas invariably resulted in rapid onset of death, which occurred characteristically in from four to twelve hours after the operation. We at first explained these deaths on the basis of the animal's unstable or inadequate carbohydrate reserves. Accordingly, the animals were routinely given dextrose subcutaneously, and sometimes intravenously, just previous to, as well as subsequent to, pancreatectomy. In spite of maintaining the blood sugar at a normal or above normal level in this manner, deaths continued to occur. Whereas the original pancreatectomies were done with the animals under pentobarbital sodium anesthesia, we changed to ether anesthesia so that the recovery of the animals could be followed to better advantage and the

be seen through the thinned cortex. After appropriate fixation, when the gland was cut through its middle, the thinning of the cortex was verified, as is evident in the photograph of the adrenal gland of dog 4Br in figure 2.

A goodly number of these preparations were lost within periods ranging from two to ten months after hypophysectomy in apparent crises of adrenal insufficiency. These crises were first precipitated by accident, and later purposely by (1) changing from one brand of dog meal to another having a lowered salt content and (2) through missing a meal when the attendant failed to feed by force an animal that refused to eat spontaneously. In these instances, the giving of dextrose failed to prevent or relieve the crises.

Obesity.—These preparations invariably exhibited definite obesity a few months after hypophysectomy. The obesity became progressively more marked as the postoperative period increased (dog 219Br, fig. 9). We did not keep one of these preparations long enough for the weight curve to form a plateau, as in the instance illustrated for dog 7Br in figure 9. The earliness of onset and the ultimate magnitude of obesity were undoubtedly enhanced by (1) the sugar taken in the drinking water during the immediate postoperative period and (2) the accompanying decrease in energy metabolism which occurred, but to a submaximal degree in the near-total hypophysectomized preparations.

TOTAL HYPOPHYSECTOMY

Without Anatomic Infringement on the Hypothalamus.—When all the hypophysial tissue was removed without detectable gross infringement on the hypothalamus (line 5, fig. 3), the animals recovered uneventfully and

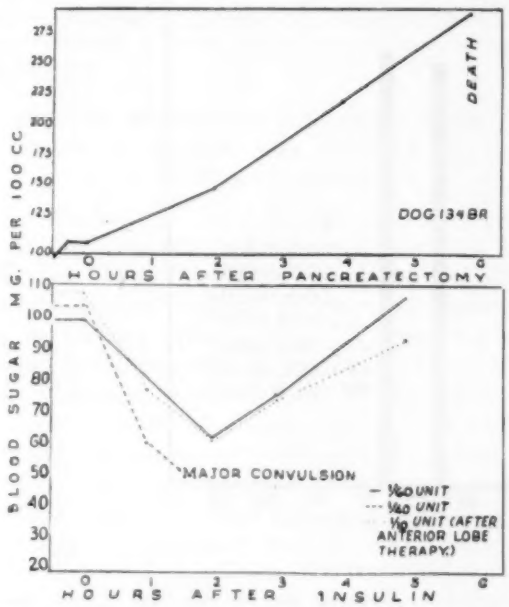

Fig. 8.—Blood sugar curve of dog 134Br (a near-total hypophysectomized preparation) showing (1) a post-hypophysectomy decrease of insulin tolerance of at least sixty times, which was returned to near-normal by appropriate administration of anterior pituitary extract, and (2) the failure of the anteror pituitary extract therapy and administration of dextrose (subcutaneous) to delay or prevent the onset of death in a hypophysectomy-pancreatectomy crisis.

appeared outwardly normal for from three to six weeks. During this time they were very susceptible to hypoglycemic crises, and the usual special care was necessary to keep such crises from precipitating. Some of these preparations were lost in hypoglycemic crises in spite of this special care. In most of the animals which survived, in from three to six weeks a characteristic crisis appeared more or less abruptly. The stools became loose, food was vomited, and muscular weakness and general lassitude set in. Mild to severe hypoglycemia was present, and, on the basis of not too reliable determinations, blood sodium was abruptly lowered. Maintaining the blood sugar at or above normal by giving dextrose subcutaneously or intravenously did not alter the appearance or course of these crises. In a few instances crises were successfully alleviated by administration of adrenal cortex extract (Upjohn) and desoxycorticosterone acetate (Ciba's Percorten). At autopsy striking atrophy of the adrenal cortex was always present, even as early as four weeks after hypophysectomy (dog 40Br, fig. 2). It was concluded, therefore, that these crises were due predominantly to adrenal insufficiency.

A few of these preparations were maintained to chronicity without therapy other than the frequent feeding regimen. In general, they behaved functionally as the near-total hypophysectomized preparations. Dog 290C, described in our paper on the relation of the pars nervosa to water metabolism,[9] is the outstanding example of this group. Its insulin tolerance was decreased only ten times (simulating that of the ordinary hypophysectomized animal), yet the adrenal glands were markedly flattened and almost black, and the cortex was markedly thinned (simulating that of a near-total hypophysectomized animal). The dog was markedly obese (fig. 9). The fat over the dorsum of the hips measured 3 cm. in depth. There was no fragment of glandular tissue in the fossal scar tissue of this animal!

With Anatomic Infringement on the Hypothalamus.—After recognizable anatomic encroachment on the hypothalamus, the physiologic status of the animals, with respect to carbohydrate metabolism and adrenal function and size, ranged through the entire number of functional categories that were encountered following the varying degrees of hypophysectomy described in the foregoing pages.

A near-maximal functional reversal is illustrated in dog 68Br, described and illustrated in the introduction of this paper. This dog had only a small anatomic defect in the ventroanterior part of the hypothalamus in addition to the hypophysectomy. A very small glandular remnant was found in the fossal scar tissue, but serial sections of the bone of the sella turcica and the soft tissues of the dorsal part of the nasopharynx revealed no extracranial hypophysial tissue. The animal's adrenal glands were not strikingly atrophied, and there was no evidence of adrenal insufficiency at any stage in the postoperative period. Deviation from the normal in insulin tolerance was negligible (only two times). The deviation from the usual after removal of the pancreas was in the opposite direction from a Houssay effect.

A functional reversal effect intermediate between dogs 290C (mild reversal) and 68Br is illustrated in dog 142Br, which had a moderate-sized anatomic defect in the ventroanterior part of the hypothalamus. Its adrenal glands were moderately atrophied. There was a small bit of tissue in the dural fossa which may have been glandular but which could not definitely be identified as such. The insulin tolerance was decreased approximately forty times, and following pancreatectomy a typical 2 plus Houssay effect obtained.

Another mild functional reversal effect is illustrated in the instance of dog 16Br. This dog had a slight anatomic infringement on the hypothalamus in addition to total hypophysectomy. This is shown in figure 5 of the article by one of us (Keller [9]) just cited. Functional encroachment on the hypothalamus was evidenced by permanent residual diabetes insipidus, which is illustrated in figure 7 of the article just cited. A functional reversal affecting adrenal function and size was evi-

9. Keller, A. D.: Endocrinology **30**:408, 1942

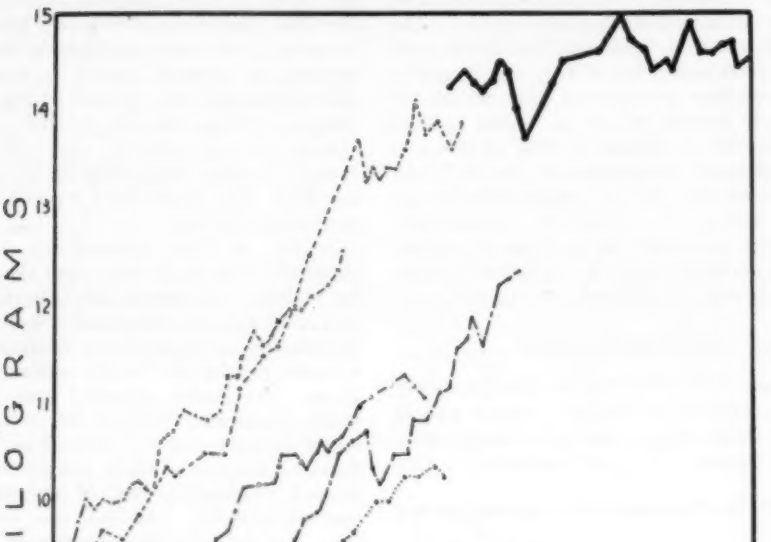

Fig. 9.—Weight curves of dogs having varying degrees of hypophysectomy.

Dog 81Br was a near-ordinary hypophysectomized preparation. This dog was fed 450 Gm. of wet Baloration meal daily for three weeks after operation. The intake was then reduced to 350 Gm. per day for two months, and then further reduced to 300 Gm. per day for the succeeding three months, at which time pancreatectomy was executed.

Dog 198Br was an ordinary hypophysectomized preparation. This dog was fed 300 Gm. of wet Baloration meal daily throughout the posthypophysectomy period.

Dog 219Br was a near-total hypophysectomized preparation. She was fed 300 Gm. of wet Baloration meal daily for three months following hypophysectomy. The intake of food was then reduced to 250 Gm. daily for two months, and then raised to 300 Gm. daily for the succeeding four months, at which time pancreatectomy was executed.

Dog 290C was an example of an animal with an anatomically uncomplicated total hypophysectomy. This dog was fed 330 Gm. of wet Baloration meal daily throughout the posthypophysectomy period.

Dog 44Br had undergone a total hypophysectomy plus considerable anatomic encroachment on the hypothalamus. This dog was fed 400 Gm. of wet Baloration meal daily for seven months following operation. The intake was then reduced to 300 Gm. daily for a period of two weeks and then raised to 350 Gm. daily for the next three months, at which time pancreatectomy was executed.

Dog 7Br had only a large destructive lesion in the anteroventral part of the hypothalamus. This dog was fed 325 Gm. of wet Baloration meal daily for four months following operation. She was placed out in the yard for the next five months, during which time her food consumption was not measured. The food intake was 300 Gm. a day for the next ten months, and then was raised to 400 Gm. a day for the next six months. It was then reduced to 300 Gm. daily for the next nineteen months. The weight curve did not vary after it reached a plateau.

Dog 12H had undergone a selective removal of the hypophysial stalk. She was fed 300 Gm. of wet Purina meal daily throughout the postoperative period.

denced by the fact that the dog lived six months, until death occurred following pancreatectomy, without special therapy. At autopsy the adrenal gland was found to be markedly atrophied but not maximally so, as shown in the photograph in figure 2. Note the size of the adrenal gland of dog 16Br compared with that of dog 4Br, a near-total hypophysectomized animal also maintained for six months. The atrophy is not so great as in the instance of dog 40Br, a totally hypophysectomized animal which died with symptoms simulating adrenal insufficiency four weeks after hypophysectomy. With respect to carbohydrate metabolism, this dog also behaved like a near-total hypophysectomized preparation. It combated successfully 1/80 unit of insulin per kilogram but was unable to combat 1/40 unit. Pancreatectomy precipitated a typical hypophysectomy-pancreatectomy crisis.

A goodly number of our animals which had small anatomic defects in the ventral or ventroanterior part of the hypothalamus in addition to total hypophysectomy exhibited no evidence of a functional reversal. These animals died early following the hypophysectomy in crises suspected of being due predominantly to adrenal insufficiency.

DEFICITS DEFINITELY ASSOCIATED WITH REMOVAL OF THE PARS ANTERIOR AS IT IS GENERALLY DELIMITED

On the basis of the foregoing data, only two of the deficits which have been described as following hypophysectomy could be reliably associated with removal of the pars anterior per se. These deficits were (1) regression of sexual functions and (2) cessation of growth. Our observations with respect to these deficits conform with the conclusions drawn by Crowe, Cushing and Homans,[4] Aschner[10] and Smith,[11] namely, that a sex or a growth deficit or both may not occur until more than two thirds of the pars anterior is removed and that more than 90 per cent must be removed before maximal deficits are always obtained. We would emphasize Smith's statement that some sexual activity may be present when far less than 10 per cent of this tissue remains intact. The fact that sexual functions are at times eliminated with as much as approximately one tenth of the pars anterior remaining behind is not surprising in view of the fact that these functions can be eliminated by a lesion confined to the hypothalamus (Bailey and Bremer[12]) or by mere section of the hypophysial stalk with a good portion of the pars anterior remaining macroscopically undisturbed (Brooks[13]). In this connection, it is important to remember that although the latter procedures do frequently eliminate sexual development and activity, they do not do so invariably (Keller and Hamilton[14]).

A maximal change in coat was also associated with removal of the pars anterior. It seems probable that this phenomenon is primarily a part of the growth deficit rather than an independent factor or a part of the sex deficit. Facts supporting the supposition that the change in coat is not a part of the sex complex are: (1) It is possible to have complete inhibition or cessation of sexual development without any change in coat, i. e., following hypothalamic lesions or following section of the hypophysial stalk, and (2) a maximal change in coat has been precipitated in near-ordinary hypophysectomized dogs in which estrus continued to occur, as judged by the outward signs of estrus.

There remain the possibility and the probability that removal of the pars anterior per se primarily affects the animal's carbohydrate reserves. In this series near-total removal or total removal of the pars anterior resulted in a definite reduction in the animal's ability to tolerate insulin. The reduction encountered ranged from ten to four times. A reduction of ten times is relatively a minor one as compared with the invariable reduction of sixty to one hundred times in near-total hypophysectomized preparations. In this connection, it is to be remembered that the over-all maximum reduction in insulin tolerance is likely due to multiple factors, such as an independent pars anterior factor, an independent pars tuberalis factor, the posterior lobe carbohydrate factor and the complication of adrenal atrophy. It is conceivable that the factor of the pars anterior per se is due directly to the absence of the growth principle. It is true that the entire pars nervosa of the pituitary body can be eliminated by isolation and atrophy without affecting the animal's reaction to insulin (Keller[15]). But this does not necessarily indicate that that factor does not enter into the over-all deficit following total hypophysectomy. The other mechanisms might be entirely adequate in maintaining carbohydrate reserves within the normal range if only the pars nervosa carbohydrate factor is lacking.

DEFICITS ASSOCIATED WITH ENCROACHMENT ON THE TISSUE OF THE HYPOPHYSIAL STALK

It was definite that when the tissue of the stalk was progressively encroached on as

10. Aschner, B.: Arch. f. d. ges. Physiol. **146**:1, 1912.

11. Smith, P. E.: Anat. Rec. **52**:191, 1932.

12. Bailey, P., and Bremer, F.: Arch. Int. Med. **28**: 773, 1921.

13. Brooks, C. M.: Am. J. Physiol. **121**:157, 1938.

14. Keller, A. D., and Hamilton, J. W., Jr.: Am. J. Physiol. **119**:349, 1937.

15. Keller, A. D.: Proc. Soc. Exper. Biol. & Med. **42**:837, 1939.

schematized in figure 3 and illustrated in figure 4—subsequent to the removal of the pars anterior and the posterior lobe—the deficits in carbohydrate reserves, in function and size of the adrenal cortex and in fat metabolism were progressively increased, whereas the Houssay phenomenon and the hypophysectomy-pancreatectomy crisis followed encroachment of a restricted nature.

In all of these instances a question which comes much into the foreground is whether these deficits are due to the absence of tissue limited to the stalk or whether the functions concerned are carried by elements common to both the pars anterior and the pars tuberalis. On the basis of preliminary observations it is essentially certain that the latter is the case, at least in the instances of some of these functions. Irrespective of the answer to this question, the observations in their present state are clearly indicative that these functions are associated with the pars tuberalis. This finding is of fundamental importance because, so far as we are aware, no one has previously presented evidence based on the extirpation approach which has suggested a function or functions for this tissue. In this connection it is pertinent to call attention to the fact that a great deal of the investigative work that has been done relative to localizing specific functions to the pars anterior per se has been derived from the use of so-called extracts of the anterior lobe. In the preparation of these extracts there has frequently, if not usually, been a fundamental error in that they have been derived jointly from the pars anterior and the pars tuberalis.[16]

Carbohydrate Reserves.—The additional lowering of the carbohydrate reserves which is associated with progressive infringement on the tissue of the stalk is striking (an increase from ten to one hundred times). It is conceivable that this could be entirely due to the associated increased atrophy of the adrenal cortex. Certainly one would expect the adrenal atrophy to enter into the over-all picture in the chronic preparation. In our experience the appropriate administration of adrenal cortex extract (Upjohn) does return carbohydrate reserves toward normal in the near-total hypophysectomized preparation. However, we believe that the reserves are progressively lowered independently of the adrenal atrophy, because the near-total hypophysectomized dog is immediately extremely susceptible to hypoglycemic crises, whereas the tendency toward crises of adrenal insufficiency does not appear until three weeks after the hypophysectomy, and maximal anatomic atrophy of the adrenal cortex does not obtain for four or more weeks.

Our results did demonstrate emphatically that the predominant lowering of carbohydrate reserves was not due to the absence of the acidophils of the pars anterior as has recently been surmised by Heinbecker and Rolf.[17]

Houssay Effect.—It is to be noted that the Houssay effect was encountered only in the presence of a sizable but restricted encroachment on the tissue of the stalk. At the same time, we are essentially certain that total removal of the pars anterior was successfully executed, in the presence of which there was no noticeable evidence of a Houssay effect following pancreatectomy (dog 184Br, fig. 5).

In connection with the study of the Houssay phenomenon, we would point out that great care must be taken to make sure that there is total removal of the pancreas in the preparations in which an attempt to assay the magnitude of the phenomenon is being made. In the presence of a mild Houssay effect, a pancreatic remnant which in a normal animal would not produce a noticeable lessening of the diabetes mellitus would definitely distort the magnitude of the effect.

We would also emphasize that the survival time following pancreatectomy is not too reliable a criterion for evaluating the extent of a Houssay effect, as it applies primarily to carbohydrate metabolism. The fact that the magnitude of the amelioration of the diabetes mellitus is not correlated with the survival time was pointed out by Houssay in his original paper (Houssay and Biasotti.[18] The animal that lived longest among his original 9 animals showed the most intense hyperglycemia, whereas the animal that survived the shortest interval of time exhibited the least hyperglycemia. The dextrose tolerance test has been found to be more reliable than survival

16. These statements are based on the use of "pars anterior" and "anterior lobe" as synonymous terms. This nomenclature is the one generally used, or at least implied, and is the one recommended by D. M. Rioch, G. B. Wislocki and J. L. O'Leary in their recent overhauling of hypophysial terminology (A. Research Nerv. & Ment. Dis., Proc. **20**:3, 1940). The work reported here strongly supports the nomenclature used by Best and Taylor in their current textbook; namely, the term "anterior lobe" is used to include the combined tissues of the pars anterior and the pars tuberalis. When "anterior lobe" is used in this way, "anterior pituitary extracts" are correctly described for what they are.

17. Heinbecker, P., and Rolf, D.: Am. J. Physiol. **141**:566, 1944.

18. Houssay, B. A., and Biasotti, A.: Arch. f. d. ges. Physiol. **227**:664, 1931; Endocrinology **15**:511, 1931.

time for assaying the status of the disturbance in carbohydrate metabolism (fig. 7).

On the basis of the color of the liver in the gross specimen and ordinary hematoxylin and eosin staining of sections of liver, the evaluation of the accumulation of fat in the liver is also a reliable assay tool for determining the presence of a near-maximal Houssay effect. It is also believed that this can be used in evaluating a submaximal effect if the postpancreatectomy survival time is rigidly standardized. In the presence of a maximal or near-maximal effect the standardization of survival time is not necessary for the result to be obvious. In our animals which exhibited a near-maximal Houssay effect with respect to the presence of a fatty liver, the external pancreatic secretions were absent, and there were no dietary factors that deviated from those present in our control animals. It seems clear that Dragstedt's failure (Julian and associates [19]) to encounter a reduction of fatty infiltration of the liver in his hypophysectomized-pancreatectomized animals as he suspected he would was due to the fact that he did not have animals in his series which exhibited sufficient amelioration of the disturbance in fat metabolism to be detected without standardizing the survival time. We would stress the fact that in dealing with this problem the term "hypophysectomized-pancreatectomized preparation" cannot be used synonymously with "Houssay animal." For a "Houssay animal" to obtain, amelioration of pancreatic diabetes must have been demonstrated.

This observation is compatible with Dragstedt's contention that the pancreas elaborates a fat metabolism autacoid (lipocaic), the absence of which is in large part responsible for the fatty infiltration of the liver following pancreatectomy. This is particularly true, because we have maintained Houssay animals several months without fatty livers developing. Assuming that this is true, one perceives clearly that the action of lipocaic is closely interrelated with the action of insulin, because fat accumulates in the untreated pancreatectomized dog's liver more rapidly than in the insulin-treated pancreatectomized dog's liver, and insulin itself clears the acetonuria of diabetes mellitus.

Hypophysectomy-Pancreatectomy Crises. — We have repeatedly and invariably observed that a chronic near-total hypophysectomized animal does not survive pancreatectomy for more than a few hours. This has been disappointing because in the light of the recently discovered Houssay effect we expected to encounter the very maximal amelioration of diabetes mellitus under these circumstances. It was thought that such an animal would not show even so much as raised dextrose tolerance and would survive the pancreatectomy indefinitely, i. e., that the pancreatectomy would not alter the near-total hypophysectomy picture in any way.

It was at first suspected that the deaths were due to crises of hypoglycemia or adrenal insufficiency precipitated by the stress of the pancreatectomy. These possibilities have been investigated to the extent that the combined administration of carbohydrate, anterior pituitary extract, adrenal cortex extract and desoxycorticosterone under the conditions and dosages stated previously did not protect these animals from the hypophysectomy-pancreatectomy crises. The only criterion of the efficacy of the pituitary and adrenal cortex extracts used was that they both were effective in returning the animal's carbohydrate reserves to near-normal. (See figure 8.) This safely rules out the carbohydrate factor, but there still remains some question as to whether hypoadrenalism is not responsible, because the clinical aspect of the animals in the hypophysectomy-pancreatectomy crises seems to be identical with the shock syndrome which follows intestinal manipulation in adrenalectomized dogs as described by Swingle and associates.[20]

If these crises prove to be due specifically to hypofunction of the adrenal glands and accordingly are peculiar only to the chronic near-total hypophysectomized preparation, the occurrence of such a crisis would be an admirable experimental test for the presence of "subclinical" hypoadrenalism.

Adrenal Function and Size.—The relation of the extent of encroachment on the tissues of the hypophysial stalk to the degree of atrophy of the adrenal cortex was particularly striking. Although mild atrophy of the cortex probably occurred in instances following total removal of the pars anterior (ordinary hypophysectomy), it is questionable whether any atrophy occurred when sufficient stalk tissue remained. In the light of Smith's [11] work on rats it is suggested that there is a difference in adrenal over-all size due specifically to the absence of the growth principle. If such a relationship exists, it is slight. Smith obviously encountered a failure of adrenal atrophy to parallel dwarfing and sexual regression following varying degrees of removal of the pars anterior. "No structural abnormal-

19. Julian, O. C.; Clark, D. E.; Proback, J. V.; Vermeulen, C., and Dragstedt, L. R.: Am. J. Physiol. **138**:264, 1942.

20. Swingle, W. W.; Parkins, W. M.; Taylor, A. B., and Hays, H. W.: Am. J. Physiol. **124**:22, 1938.

ities of the thyroids and adrenals were distinguished even in those animals which had the smaller amounts of pars anterior."

Striking atrophy of the adrenal cortex invariably occurred in the presence of near-total hypophysectomy (dog 4Br, fig. 2). The atrophy in this situation was not maximal, for sufficient cortical function remained to carry the animals indefinitely under a protective feeding regimen, i. e., without specific therapy. Nevertheless, adrenal cortical function was near its lower limit, for what appeared to be crises of adrenal insufficiency were precipitated in some of these animals by reduction of salt in the diet.

In preparations in which all of the tissue of the stalk was removed (total hypophysectomy), the adrenal cortex atrophied markedly and rapidly to such an extent that in three to four weeks' time the animals died in crises suggesting adrenal insufficiency. (Note the extreme atrophy of the cortex of the adrenal gland of dog 40Br in figure 2. Also note that the cortex is considerably thinner than that in dogs 4Br and 16Br.)

It is significant, we believe, that the function of the adrenal cortex continues during the time atrophy is occurring. This is quite in contrast to the situation with carbohydrate metabolism. Animals are even more prone to hypoglycemic crises during the days immediately following an appropriate type of hypophysectomy than after they have attained the chronic state.

Obesity.—Several years ago we called attention to the fact that in certain instances in which obesity followed hypophysectomy and hypothalamic lesions it was accompanied with polyphagia.[21] That the polyphagia and the resultant obesity were due indirectly to increased sensitivity to insulin was investigated and ruled out (Keller[15]). The question was raised as to whether obesity in these cases, in which the food intake was not limited, was simply a matter of overeating or whether there was in addition an underlying disturbance in fat metabolism. In order to rule out overeating as a variable in this study, the animals were maintained on a constant restricted daily food intake which did not produce obesity in otherwise normal animals under the same cage regimen (dog 12H, fig. 9). The matter of undereating was ruled out by forced feeding when the food was not taken spontaneously. Accordingly, we interpret the appearance of obesity in these animals as definitely indicating a disturbance in fat metabolism of a nature that actually enhances the laying down of fat in the various fat depots (pathologic obesity in the true sense of the term). This would particularly apply to (1) animals weighing over 6 Kg. when 300 Gm. of food was the maximum given and (2) animals in which an increase in energy metabolism occurred concurrently with the development of obesity.[22] In using this method, it is necessary to maintain the animals for a long period after the hypophysectomy in order to make certain whether obesity is going to appear. Naturally, on a restricted food intake the rate of onset of obesity and also the maximal obesity attained would be limited.

It should be noted that in attempting to assay the presence and the extent of obesity, factors other than weight curves must be appropriately considered. The general appearance, the thickness of the subcutaneous fat layer and the amount of intra-abdominal fat present at autopsy are all reliable qualitative assay factors. These taken collectively, coupled with weight curves, have given us the impression that the obesity precipitated in hypophysectomized preparations is actually much greater than is reflected in the weight curves. Some of these animals begin to appear obese before the weight curves begin to rise. This must be due to an actual decrease in the over-all weight of the nonfat tissues of the animal, due presumably to the absence of the growth factor. It has been found that general appearance is the more reliable single criterion for the estimation of the presence or the absence of obesity. For instance, laymen will immediately point out the fat animals as against the nonobese ones which are, nevertheless, in a well rounded physical condition.

Although obesity did definitely follow an ordinary or even a near-ordinary hypophysectomy, we are not certain that it always does so. In the instances in which obesity was not definite, the animals were not maintained sufficiently long after the operation to make certain that obesity would not appear. It will be noticed in the growth curves in figure 9 that under the regimen of a restricted intake of food the onset of obesity is slow but progressive over at least a year's

21. Keller, A. D.; Hare, W. K., and D'Amour M. C.: Proc. Soc. Exper. Biol. & Med. **30**:772, 1933. Keller, A. D., and Noble, W.: Am. J. Physiol. **113**:79, 1935. Keller, A. D., and Noble, W.: ibid. **116**:90, 1936.

22. Dog 7Br remained obese in spite of a restricted intake of food and a marked postoperative increase in its basal energy metabolism. This paralleled the increase in total body weight (Bruhn, J. M., and Keller, A. D.: Am. J. Physiol. **133**:229, 1941). In addition, after the obesity curve formed a plateau, a raising of the intake of food for a period of twenty-four weeks did not increase the magnitude of the obesity. The dog's tolerance for insulin was normal after the hypothalamic lesion.

time. Many of these preparations were terminated previous to six months, and accordingly their obesity and their weight curves had not progressed sufficiently to indicate more than a trend. The impression was gained that obesity in near-ordinary and ordinary preparations was more obvious in young animals than in adult animals, as was pointed out by Cushing and by Aschner.[10] In certain cases, the obesity appeared to develop as rapidly and to be as pronounced as that encountered in other animals as to which there was no question of the obesity's being near the maximum encountered by us. This is illustrated by the weight curves for dogs 81Br and 198Br, shown in figure 9. Dog 81 Br was a near-ordinary hypophysectomized preparation and was an adult dog in good physical condition at the time of the hypophysectomy. Dog 198Br was an ordinary hypophysectomized preparation.

We invariably encountered striking obesity or an indication of it in the near-total hypophysectomized preparations which were maintained for as long as six months after the hypophysectomy (dog 219Br, fig. 9). In certain instances the obesity was as marked as that which was encountered following total hypophysectomy plus infringement on the hypothalamus (dog 44Br, fig. 9) or following lesions which were confined to the hypothalamus (dog 7 Br,[22] fig. 9).

On the basis of preliminary experiments and the aforementioned control material it is definite that obesity is not routinely precipitated by section of the hypophysial stalk alone or by selective removal of hypophysial stalk tissue (the tissue between lines *1* and *4*, fig. 3). The weight curve for dog 12H (stalk removed) in figure 9 is representative. Evidence that pars anterior tissue remained functionally intact was attested to in this particular dog by the fact that subsequent to the removal of the stalk, the animal exhibited estrus, mated, whelped and reared a normal litter of puppies. Evidence of success in the surgical procedure was attested to by an intact surgical specimen. The animal is still living.

These observations strengthen considerably our conviction, established several years ago, that the hypophysis per se is intimately associated with fat metabolism. Our observations verify in all details Cushing's original observations and conclusions. If the obesity that follows hypophysectomy alone were due to a neighborhood derangement in hypothalamic functions, obesity should be precipitated in the same incidence by section or removal of the stalk. Such a result definitely does not occur. If obesity following hypothalamic puncture were due to the elimination of neurogenic impulses passing from the hypothalamus to the hypophysis, section of the stalk should precipitate obesity. This also is definitely not the case. We are well aware that obesity following hypophysectomy per se is currently explained on the combined basis of reduced basal energy metabolism and increased intake of food (polyphagia). There is no doubt that a decrease in energy metabolism is a factor enhancing the obesity of near-total hypophysectomized preparations. One of the reasons for our belief that this is not the only factor is that the decrease in energy metabolism in these preparations was less than that which was present in the dogs which had undergone total hypophysectomy with infringement of the hypothalamus. Further, the energy metabolism of near-ordinary and ordinary hypophysectomized preparations does not deviate perceptibly from the normal (Bruhn and associates[3b]), yet obesity develops at least in some of these preparations. The obesity in these instances cannot be distinguished from that precipitated in instances of hypothalamic puncture, in which there can be no doubt that the obesity is truly pathologic.

In this connection, at the expense of repeating, we stress that it is fully recognized that fat metabolism is not solely a hypophysial function, because it is well established and it is our experience that maximal obesity can be precipitated also by (1) a hypothalamic lesion wherein the adenohypophysis is left macroscopically undisturbed and (2) surgical encroachment on the hypothalamus subsequent to either section of the stalk or total hypophysectomy.

THE PROBLEM OF WHY IN SOME INSTANCES CERTAIN OF THE HYPOPHYSECTOMY DEFICITS FAIL TO APPEAR FOLLOWING ATTEMPTED TOTAL HYPOPHYSECTOMY

The data collected in this study have not clarified with certainty the problem of determining which of the two variables—functional hypothalamic involvement or an unusually selective and potent activity of a very small glandular remnant—was responsible for the failure of certain of the hypophysectomy deficits to precipitate (reversal effects described in the introduction to this paper). However, as a result of this over-all study, we are more inclined to think seriously in terms of the hypothalamic involvement as being the responsible variable. The minute multiglandular remnants found in the fossal scar tissue seem altogether inadequate for the maintenance of the normal or near-normal functions which were in evidence. The fragments were no larger than the one shown in

figure 4 E at the end of arrow *2*. More important is the fact that we have never encountered any tendency toward a reversal effect in near-total hypophysectomized preparations, even in the presence of much larger fossal glandular remnants than those in the presence of which we encountered maximal reversals in total-plus preparations.

We have naturally speculated considerably as to the nature and the physiologic importance of such a possible hypothalamic mechanism. The reversal effects could be explained by postulating that hypothalamic tissue elaborates endocrine principles having actions opposite to the known hypophysial principles.[1] On such a basis, the normal end result in the intact animal is maintained by the proper balancing of two antagonistic endocrine principles, as reciprocal innervation obtains in the central nervous system. This concept is to some extent embodied in the doctrine of antihormones. It should be borne in mind, however, that the contraprinciples act directly on the same "target" mechanism as the hypophysial autacoids rather than by inactivating these autacoids. Accordingly, the contraprinciples continue to exert their actions in the absence of the corresponding antagonistic hypophysial principles.

On the basis of this hypothesis, the atrophy of the adrenal cortex following an uncomplicated total hypophysectomy would be due to the continued activity of the contra-adrenotropic principle in the absence of the counterbalancing adrenotropic principle (dog 40Br, fig. *2*). In the event that both the adrenotropic principle and the contra-adrenotropic principle were simultaneously eliminated (total hypophysectomy plus drastic functional infringement on the hypothalamus), adrenal atrophy would not occur; i. e., the cortex would maintain status quo. This would represent a reversal effect of the magnitude encountered in dog 5Br (fig. *2*). In the event that the contra-adrenotropic principle was only partially impaired, there would be corresponding partial atrophy of the adrenal cortex (dog 16Br, fig. 2). This would represent a mild (dog 16Br) or moderate (dog 142Br) reversal effect. In the event that the contra-adrenotropic principle was eliminated or impaired without there being a corresponding impairment of the mechanism elaborating the adrenotropic factor, there would be corresponding hypertrophy of the adrenal cortex and a tendency for hyperadrenalism or Cushing's syndrome to develop. Actually we have encountered what we suspected were abnormally large adrenal glands in animals which had lesions confined to the hypothalamus and were maintained for a long period.

The concept of contraprinciples (either of hypothalamic or of hypophysial origin) is not only made tangible by the results which we encountered experimentally—with respect to carbohydrate metabolism and adrenal function and size—but also by clinical data which have always baffled the physiologist as well as the clinician. A good many clinical entities—precocious sexual development, diabetes mellitus, overgrowth, hyperadrenalism, hyperthyroidism and others—long suspected on occasion as being due to derangement in the basal region of the brain would be readily explained if such a state of affairs actually existed.[23]

23. There are instances on record in which at autopsy the hypophysis was found to be within the normal range when clinically there was every reason to suspect that this gland had been functionally hyperactive. This has been shown particularly well by H. Cushing and L. M. Davidoff (The Pathological Findings in Four Autopsied Cases of Acromegaly, with a Discussion of Their Significance, Monograph 22, Rockefeller Institute for Medical Research, 1927, pp. 1-121). The reader is likely to find the following reprinting of the "Summary and Comment" from their "Case I" provocative reading in association with the herein outlined concept of contrahypophysial principles. According to this concept, functional hyperactivity would be entirely compatible with a grossly and histologically normal-appearing adenohypophysis.

"*Summary and Comment.* In this patient the disease was slowly progressive for nearly 30 years; he became increasingly incapacitated and died at the age of 52, apparently with a terminal bronchopneumonia. A prolonged treatment extending over many years with anterior-lobe extract in large doses gives rise to some speculation on its possible effect on the malady. The dominant complaint throughout was of persistent cephalalgia moderately relieved by recumbency. Nevertheless, *there was no evidence of enlargement of the pituitary fossa on X-ray examination during life*, and at autopsy the gland was found to be of normal size.

"The autopsy showed in addition to the general splanchnomegaly of unusual degree, a persistent thymus, a large colloid goitre, enlarged parathyroids, hypertrophy of the pancreas and greatly hypertrophied adrenals due to an adenomatosis of the cortical substance. To any one of these markedly changed glands an observer might in the 1880's have ascribed the malady with greater seeming probability than to the normal-sized hypophysis.

"One is inclined to ascribe the long duration of the malady to the small size of the adenoma, for many victims of the disease die from intracranial symptoms caused by an extension of the tumor beyond the sella. Yet there have been cases in which the disease has lasted longer. Sternberg, for example, records one of nearly 50 years' duration; Gauckler and Roussy's patient who lived to be 83 had symptoms for 38 years, the tumor having become cystic.

"What makes this Case I of paramount interest is the fact that the pituitary body was not enlarged. Certainly if Marie and Marinesco had chanced to encounter such an example of acromegaly at autopsy instead of

(Footnote continued on next page)

COMMENT REGARDING CUSHING'S INABILITY TO MAINTAIN "TOTALLY HYPOPHYSECTOMIZED" DOGS

Houssay's demonstration that Cushing's cachexia hypophyseopriva was predominantly due to the manifestation of a hypoglycemic crisis gave immediately a plausible explanation as to why Paulesco, Cushing and others were unable to maintain "totally hypophysectomized" dogs for long periods after operation. These investigators made no attempt to feed their dogs more than once a day, and when food was not taken spontaneously, they did not resort to forced feeding. On the basis of the data presented in this paper it is obvious that without special care in post-hypophysectomy feeding dogs having hypophysectomy in which the stalk is severed at any level will early succumb in a hypoglycemic crisis. A goodly number of those having the stalk cut so that the proximal tip of the pars anterior remains will also die in crises. If animals with near-ordinary, ordinary or ordinary-plus hypophysectomies are successfully carried through the immediate postoperative period, special care in feeding is no longer necessary. *Therefore a dog's long term survival following an attempted total hypophysectomy is pathognomonic of either (1) a functional infringement on the hypothalamus or (2) the presence of a potent functional glandular remnant.*

In spite of the fact that the relation between cachexia hypophyseopriva and hypoglycemic crises has been verified again and again, certain investigators and reviewers continue to make the statement that Cushing's dogs undoubtedly died as the result of neighboring damage done to the hypothalamus. We wonder if it has never occurred to these critics that this conclusion, even when made before Houssay's discovery, was inconsistent with Cushing's ability as an intracranial technician working on clinical pathologic material. Why should his intracranial technic on experimental normal material (much less difficult to deal with than pathologic material) have been suspected as being consistently poor, i. e., just opposite to the excellency of the technic in his surgical clinic? This is the implication made by critics who foster the explanation that the inability to maintain the dogs was due to hypothalamic injury. Actually, the fact that all of Cushing's dogs died in hypoglycemic crises evidences (1) the consistence of his surgical technic and (2) the fact that he neither encroached functionally on the hypothalamus nor left functional glandular remnants in the pituitary fossa. Cushing was fully aware of the possibility of complications arising from hypothalamic involvement and from the presence of glandular remnants and took unusually great care to rule out both of these factors.

SUMMARY

Varying degrees of hypophysectomy were executed in dogs. The animals were maintained for several months after being hypophysectomized. During the later stage of their survival, the status of the carbohydrate metabolism was assayed on a quantitative basis. Observations with respect to the other established hypophysial deficits were made, but not with the exactness with which carbohydrate metabolism was studied.

Sexual regression, cessation of growth and marked change in coat were the only deficits that could be reliably associated with the removal of the pars anterior per se.

The lowering of carbohydrate reserves, the occurrence and the magnitude of the Houssay effect and a decrease in adrenal function and size were associated with the progressive encroachment on the hypophysial stalk tissue, i. e.,

one in which the hypophysis was unmistakably enlarged they would have had ample grounds to oppose the view of Minkowski and others who were inclining towards the hypophysial origin of the disease. The much more obviously involved adrenals might easily have been held responsible if indeed a primary disorder of any one of the ductless glands were in those days to be considered at all as etiologically concerned in the disease. Yet a normal-sized gland has been occasionally found in bodies outspokenly acromegalic. Israel's much-quoted though incompletely recorded case (1901) may be recalled; also that of Bleibtreu (1905), probably a case of gigantism in which a degenerative process of haemorrhagic character was found replacing the hypophysis, a fact which led him to enter the lists as a champion of the hypofunction theory of acromegaly. In the same year (1905) Dean Lewis described another case with a macroscopically normal gland of which, however, it was stated that "the great increase of highly functioning chromophile cells, their irregular disposition and the lessened amount of stroma, are indicative of excessive function." In 1906, Widal, Roy and Froin reported a case in which the gland was actually atrophied and contained a small cyst, but otherwise showed nothing they regarded as abnormal. And one may recall, too, the case described by Petren (1907) over which a controversy arose: for with a normal-appearing hypophysis a coincidental syringomyelia was found which naturally led him to the view that the disease was primarily neurogenic.

"Unfortunately in our own case the process through which the hypophysis had been put for the purpose of decalcifying its bony envelope made the tissue unfit for special stains for granules, and the gland might well enough be regarded as normal. Nevertheless, with ethyl violet the protoplasm shows the coloration distinctive of the alpha granule cells even though the isolated granules are not clearly demonstrable. Moreover, in view of our present-day knowledge of the disease and belief in its unquestioned dependence upon the acidophilic elements of the pars anterior, one may feel assured from the appearance of the slides that there is an acidophilic adenoma or local hyperplasia of acidophilic elements in the centre of the pars anterior."

subsequent to total removal of the pars anterior and the posterior lobe. The greater the encroachment on the stalk the greater were these deficits. These observational facts are interpreted as indicating that the pars tuberalis normally elaborates (presumably in common with the pars anterior) the autacoids which are essential for the maintenance of these functions.

There was essentially no accumulation of fat in the livers of animals showing a near-maximal Houssay phenomenon.

Obesity occurred following (1) hypophysectomy per se, (2) lesions restricted to the hypothalamus, (3) hypophysectomy plus encroachment on the hypothalamus and (4) section of the stalk plus encroachment on the hypothalamus. The obesity which followed hypothalamic involvement was definitely pathologic and that following hypophysectomy per se is believed also to have a pathologic component. Obesity has not been encountered when the surgical procedure was limited to section of, or selective removal of, the hypophysial stalk.

In certain instances in which there was gross infringement on the hypothalamus in addition to total removal of the hypophysial tissue save for a minute fragment located in the region of the dural canal of the artery to the posterior lobe, the deficits in carbohydrate metabolism and adrenal function and size were submaximal or did not deviate materially from the normal. This observation suggests the possibility that there are elaborated in the immediate environs of the hypothalamus endocrine principles having actions contrary to the actions of certain of the known hypophysial principles.

An all-important conclusion to be derived from this study is that the term "hypophysectomy" should not be used indiscriminately. In reporting work done on hypophysectomized animals, it is rigidly requisite to describe the type of hypophysectomy attained, not only anatomically but with respect to the functional results. In the anatomic examination the same attention should be focused on the amount of stalk remaining as on pars anterior remnants.

RELATIONSHIP OF BOECK'S SARCOID AND TUBERCULOSIS

REPORT OF A CASE IN WHICH TUBERCULOSIS OF LYMPH NODES WAS ASSOCIATED WITH FEATURES HIGHLY SUGGESTIVE OF SARCOID

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Numerous arguments have been presented in support of or against the probability that Boeck's sarcoid is an atypical reaction to the tubercle bacillus or its products. Should an etiologic relationship be proved, there would be provided important material for the investigation of virulence of the organisms and the resistance of the host as factors in tuberculosis.

Acid-fast bacilli have been reported to be present in early lesions of sarcoidosis in a few instances, the bacilli later disappearing.[1] Similarly in the case to be reported, of tuberculosis involving cervical lymph nodes and the overlying skin, certain histologic and immunologic features are strongly suggestive of sarcoidosis.

REPORT OF CASE

A 9 year old Caucasian girl was seen July 30, 1943 because of sore throat of three days' duration, accompanied by noticeable swelling of the neck. During the previous year she had tired easily and had failed to gain weight, and nasal discharge had been noted. Her mother's sister had had a supposedly tuberculous cervical lymph node fifteen years previously, which was removed; otherwise no tuberculosis was known to have occurred in the family. Examination revealed a fairly well developed but thin child, weighing 64 pounds (29 Kg.). Her temperature was 100 F. The nose contained a little mucoid material, and the tonsils were enlarged and reddened. The lymph nodes of both anterior cervical chains were moderately enlarged and tender. The remainder of the examination revealed no abnormalities. With sulfathiazole therapy, the signs of acute tonsillar inflammation subsided, and the enlargement of the lymph nodes disappeared. Excision of tonsils and adenoid tissue October 14 was followed by an uneventful convalescence.

Examination on Feb. 11, 1944 revealed painless enlargement of lymph nodes of the anterior cervical groups, especially on the right, where several nodes were 1 cm. in diameter. The skin over a lymph node 1.5 cm. in diameter was purplish red. Superficial lymph nodes elsewhere were not enlarged, the spleen was not palpable, the eyes and the parotid glands were not remarkable, and the digits presented no deformities. A hemogram revealed 15.3 Gm. of hemoglobin per hundred cubic centimeters of blood and 5,000,000 erythrocytes and 10,400 leukocytes per cubic millimeter. No eosinophils were seen in the routine differential count. The urine was normal. Serum protein content, determined by two examinations, were, albumin, 5.5 Gm., and globulin, 3.6 Gm., per hundred cubic centimeters. The cholesterol content of the blood was normal. A roentgenogram of the chest revealed no mediastinal masses but disclosed three widely separated small areas of calcification in the right pulmonary field. No changes in bones, joints or soft tissues were demonstrated by roentgen examination of the hands and the feet.

At operation, the largest cervical lymph node was found to be adherent to the skin and the subcutaneous tissue and was excised with difficulty. The wound healed promptly, though a little clear fluid drained at intervals for a short time. No fluid could be obtained for inoculation into animals. The remaining enlarged lymph nodes became progressively smaller and none were palpable after the second postoperative month. The patient's oral temperature rose to as high as 99.6 F. each afternoon during the six weeks after operation, and it did not remain entirely normal until six months had passed, since which time she has been afebrile. In April 1945, she weighed 90 pounds (41 kg.) and appeared to be in perfect health.

No cutaneous reaction to tuberculin was demonstrated in tests performed a few days before biopsy and on three occasions during the following fifteen months. Each test included an intracutaneous injection of, first, 0.02 microgram, then 5.0 micrograms, of purified protein derivative of tuberculin. Old tuberculin was employed also.

Pathologic Examination.—The specimen of lymph node and overlying skin, fixed in solution of formaldehyde U. S. P., consisted of a half-dozen fragments of rather soft grayish tissue, which altogether formed a spherical mass 1 cm. in diameter. Grossly no characteristic features were evident. Microscopic examination revealed generalized, almost complete obliteration of the lymph-adenoid and cutaneous structure by fibrosis. Within several large areas of caseous necrosis were a few acid-fast rods presenting the beaded appearance characteristic of tubercle bacilli, and in the closely adjacent tissue was an occasional Langhans giant cell with peripheral nuclei. At a distance from the caseous areas, on the other hand, were numerous discrete tubercles devoid of caseation and significant lymphocytic infiltration, which were characterized in large part by a mosaic arrangement of the epithelioid cells. Some tubercles contained a very large giant cell with nuclei situated either centrally or scattered throughout the cell. A striking characteristic of a number of the giant cells was the presence of intracytoplasmic inclusions, a few of which stained deeply with hematoxylin.

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1. Nickerson, D. A.: Arch. Path. **24**:19, 1937.

The tonsils and the adenoid tissue excised in 1943 were reexamined. Macroscopic inspection of the entire specimen revealed no granulomatous lesions, and microscopic study disclosed only lymphoid hyperplasia of marked degree.

portions of the lesion were, however, typical of sarcoid: "hard tubercles" presenting a mosaic pattern and largely devoid of lymphocytic reaction, with very large giant cells of the non-

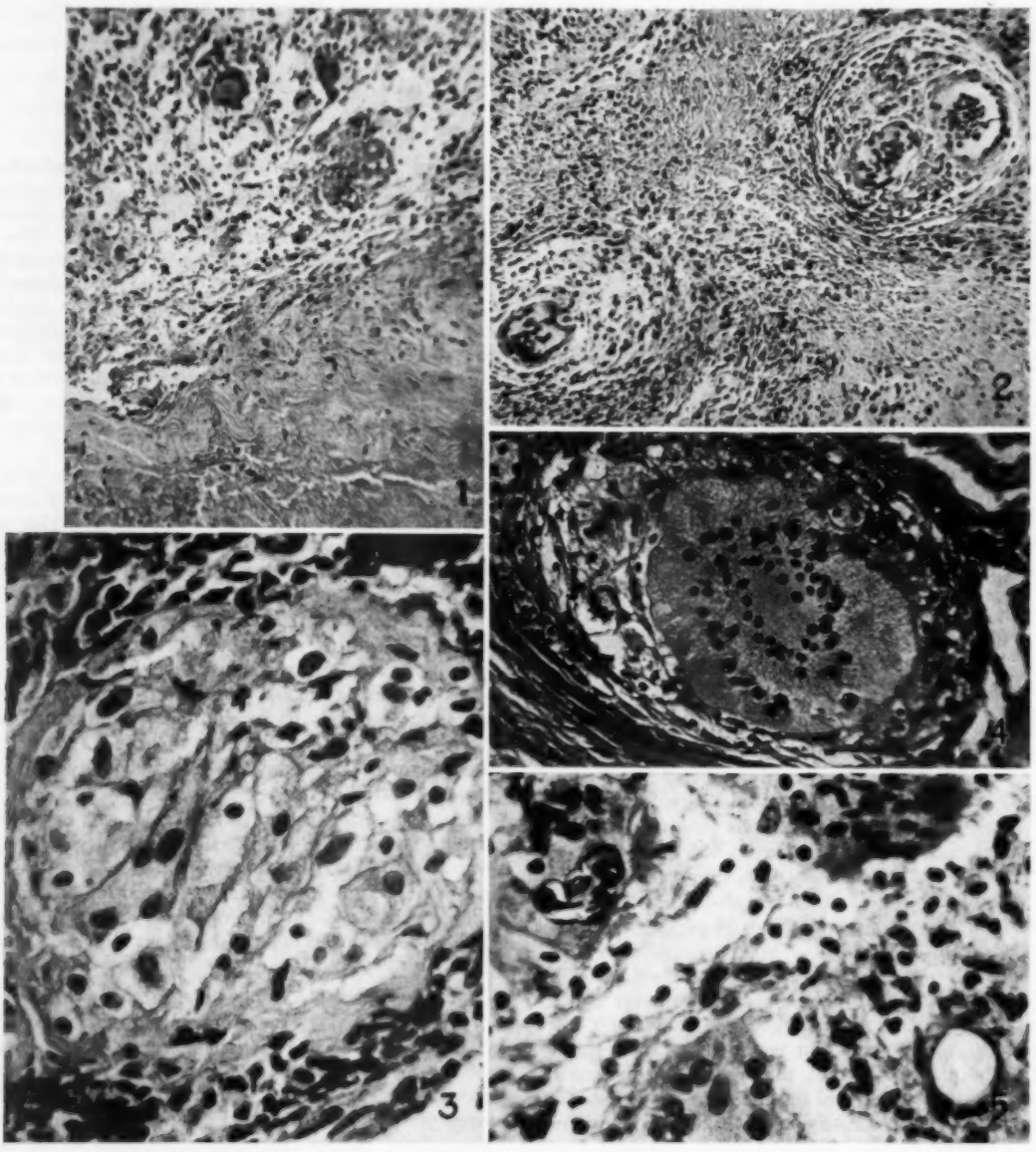

Fig. 1.—Border of a caseous area, with a typical Langhans giant cell; × 125.

Fig. 2.—Two noncaseous tubercles; × 125. The surrounding lymphadenoid tissue has undergone fibrosis.

Fig. 3.—Tubercle composed of epithelioid cells arranged in mosaic pattern; × 500.

Fig. 4.—Tubercle containing a giant cell 165 microns in diameter; × 250. The nuclei of this large cell are located in a predominantly central position.

Fig. 5.—Giant cells showing inclusions; × 500. That at the extreme left contains a large inclusion exhibiting strong affinity for hematoxylin. A faintly staining inclusion can be seen at the lower right hand corner of the photomicrograph.

COMMENT

The diagnosis of tuberculosis in this case was indicated by the presence of caseous areas containing morphologically typical acid-fast bacilli. The histologic features observed in noncaseous specific foreign body type. Of especial interest are the giant cell inclusions, some of which stained intensely with hematoxylin. These peculiar structures did not differ from those repeatedly found in typical cases of sarcoidosis and

reported as "characteristic of the disease."[2] The rigorous demand that lesions of sarcoidosis, to be acceptable, present no caseous areas or tubercle bacilli is applicable to practical diagnosis and not to experimental investigation of early lesions, in which such a dictum would stifle at the start any interrogation of the relationship of sarcoid and tuberculosis.

The patient's lack of cutaneous reaction to tuberculin suggests "anergy" to the acid-fast bacilli which were present. That tuberculous infection can occur without the development of cutaneous allergy has been demonstrated in the rat, in which tubercle bacilli incite a lesion resembling sarcoid.[3] Similarly, the rarity of tuberculin sensitivity in patients with sarcoid as compared with the general population suggests that persons in the former group react atypically to tuberculous infection. The rapid healing of the biopsy wound in this case, despite almost certain contamination by acid-fast bacilli, would seem to indicate unusually high resistance to the micro-organisms. Because unfixed biopsy material was not available, there was no opportunity to identify the bacteria fully or to test their pathogenicity or antigenicity in laboratory animals.

The patient's hyperglobulinemia is another feature characteristic of sarcoidosis.

If the lesion in this case actually does represent both sarcoid and caseous tuberculosis, one might suggest that the presence of the two diseases is coincidental or that sarcoid had predisposed to invasion by tubercle bacilli. Longcope, for example, in reporting active tuberculosis in 8 of 25 patients presenting sarcoidosis at autopsy, stated that patients with sarcoid "seem to be susceptible to tuberculosis."[2] He also mentioned, however, that the high frequency of association of the two diseases has been emphasized by those who believe sarcoid is merely a peculiar form of tuberculosis. In the case presented in this report it seems probable that tubercle bacilli were responsible for both types of tissue reaction, one of which was indistinguishable from sarcoid. Hence, the observations suggest that sarcoidosis, as claimed by certain investigators, is in some instances, at least, an atypical response to tubercle bacilli or their products. That such a relationship conceivably could exist, even though bacilli usually are not demonstrable in the lesions of sarcoidosis, is substantiated by the occurrence of the organism-free "-id" reactions of leprosy and mycotic infections and by the generally conceded relationship of streptococci to rheumatic fever and glomerulonephritis.

2. Longcope, W. T.: J. A. M. A. **117**:1321, 1941.
3. Harrell, G. T.: Arch. Int. Med. **65**:1003, 1940.

SUMMARY

Tuberculosis of a cervical lymph node was found in a patient exhibiting no cutaneous reaction to tuberculin. Portions of the lesion presented caseation with acid-fast bacilli; in other areas, however, the histologic picture was indistinguishable from that of Boeck's sarcoid. It is felt that this case lends additional weight to the argument that sarcoidosis in some instances, at least, is an atypical reaction to tubercle bacilli or their products.

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THE DIVERTICULA (LUSCHKA'S CRYPTS) OF THE GALLBLADDER

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The peculiar outpouchings of the mucosa of the gallbladder known as Luschka's crypts have been so frequently described that there would appear to be nothing important that could be added to knowledge of their morphology, pathogenesis or complications. However, closer study reveals so many misconceptions concerning them that an attempt to clarify their anatomic and pathologic significance is fully justified.

HISTORICAL REVIEW

As early as 1666 Malpighi [1] mentioned small miliary glands in the gallbladders of hogs and sheep, which he thought secreted the bile found in the gallbladders. He stated that they were like a "shrunken gallbladder" and had a branched structure, and although he had never been able to find the hidden glands under the tunic (mucosa), he believed that some existed there.[2] Thirty years later Ruysch [3] noted and illustrated glands which were distributed throughout the gallbladder, not only in that part adjacent to the liver but also in the portion opposite the liver, as well as in the neck of the gallbladder and the ducts. He said that authors had frequently made mention of these glands but as yet had given insufficiently accurate descriptions of them. They were to be found in the gallbladder of man as well as in that of the swine and that of the ox, though not as numerous, as large or as readily identifiable in the first. Bianchi,[4] in 1725, described these glands in more detail and followed Malpighi and Ruysch in the use of the term "miliary." He noted that some were larger than others.[5] Muleur,[6] whose historical review of gallstone disease, published in 1884, is the most complete ever written, stated that Tacconi,[7] in 1740, observed a human gallbladder in which the middle coat was occupied by bean-shaped glands such as Malpighi had first described.

In 1751 Théophile de Bordeu,[8] an anatomist of Pau, published a book entitled *Recherches anatomiques sur la position des glandes et sur leur action.* In this work he discussed almost every gland in the animal body, including so-called lymphatic glands and even the brain. He recognized the liver as a gland and the fact that the bile underwent definite changes in the gallbladder and that gallstones were found there. But not one word was written about any glands or related structures in the walls of the gallbladder or the bile ducts. He revealed great familiarity with the writing of Malpighi, Ruysch and Bianchi but evidently was not familiar with any of their statements about glands in the gallbladder.

In the works of Vicq d'Azyr,[9] published by Moreau in 1805, appears a short chapter entitled *Sur les glands de la vesicule du fiel* (On the glands of the gallbladder). Vicq d'Azyr noted

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1. Malpighi, M.: De viscerum structura exercitatio anatomica: Accedit dissertatio ejusdem de polypo cordis, Bononiae, J. Montii, 1666, pp. 145-146.

2. Malpighi apparently was the first to identify "glands" in the gallbladder, and some later writers wrote of these as the "glands of Malpighi." Theoretically, therefore, his name might be added to those of Rokitansky, Aschoff and Luschka as a designation suggested for the structures which form the subject of this paper.

3. Ruysch, F.: Epistola anatomica, problematica quinta: De vasis sanguiferis periostii tibiae, ut et viis, per quas vesicula fellea sarcinam acquirit, Amstelodami, apud Janssonio-Waesbergios, 1725. (Ruysch's reply at Amsterdam to Gerard Frentz in 1696.)

4. Bianchi, G. B.: Historia hepatica, in hac tertia editione, numeris tandem omnibus absoluta: Seu theoria ac praxis omnium morborum hepatis et bilis, cum ejusdem visceris anatome pluribus in partibus nova; adjectis dissertationibus aliquot, aëneis tabulis, accuratis earum explicationibus, et animadversionibus ad hocce explendum opus facientibus; amplisque omnium rerum indicibus. Geneva, G. de Tournes et filios, 1725, vol. 2, pp. 977-978.

5. The translation from the Latin on which this account is based was supplied to us by Dr. Max H. Fisch.

6. Muleur, G.: Essai historique sur l'affection calculeuse du foie depuis Hippocrate jusqu'à Fourcroy et Pujol (1801-1802), Thesis, Paris, no. 320, 1884.

7. Tacconi, cited by Muleur.[6]

8. de Bordeu, T.: Recherches anatomiques sur la position des glandes et sur leur action, Paris, G.-F. Quillau, 1751.

9. Vicq d'Azyr, cited by Holmes.[63]

that these glands were more developed in quadrupeds than in man, and he referred to the descriptions of Ruysch and Bianchi. He further stated that while Santorini gave only a few details about them, he [Santorini] called them "pori mucifori" (pores giving rise to mucus). Vicq d'Azyr also referred to Winslow, who called them "lacunae," to Haller, who was convinced of their existence even though they could not always be seen, and finally to Sabatier, who found "pores" more numerous in the neck of the gallbladder than in other parts of it. Vicq d'Azyr himself attempted to inject the glands of the human gallbladder and by aid of a strong lens saw rounded bodies about the size of a pinpoint, with tiny orifices. He did not agree with van Swieten's hypothesis that they secreted bile, as he found only mucus in them. However, he thought there were formed in them peculiar concretions, which differed from ordinary gallstones because they did not float in water and were not inflammable. He said Bennet described a gallbladder which contained in its wall small round tumors and he also had seen a similar one.

In 1773 Galéati [10] gave details about a human gallbladder in which he felt small hard bodies ("corpuscles") in the wall. After an incision, by gentle pressure he expressed a mass the size of a lentil, similar in substance and color to the central core of one of the four gallstones present in the lumen. All of the hidden calculi were enclosed in a special follicle formed by folding of the same membrane which composed the "lenticular cellules." Galéati concluded that these stones were formed by solidification of the bile in the glands described by Malpighi.

In the thirty-seventh "letter" of *De sedibus et causis morborum*, published in 1769, Morgagni [11] noted, among many other details, that Gendrotsius spoke of calculi in the gallbladder, "enclosed in a peculiar membrane." Morgagni added: "and you will suppose them to have been generated in the glands of the gall bladder, and that being increased therein, they had extended their situation between the coats, in which situation these glands, also, naturally lie." He further called attention to the fact that small biliary calculi had formerly been found and demonstrated by him in these glands, "which opened by very evident orifices." He referred to an observation by the "celebrated Galéati," similar to his except that "orifices did not appear." Morgagni also recalled observations of Greiselius and of Ellerus in which gallstones were noted in the coats of the gallbladder and concluded that such calculi, "concreted in the fundus of the gallbladder and surround by a membrane produced from the linings of the gallbladder," had been formed in one of the glands. Finally in letter XV he described a gallbladder which contained eight calculi and had about ten other stones in its wall which were "not larger, in their size, than the granules of millet-seed."

Wiedemann,[12] in 1797, published a short account of a postmortem examination of the body of a man 30 years of age, in which he found attached to the inner surface of the gallbladder an elongated mass, about 4 cm. long and 1.5 cm. broad, which on section contained a whitish cheeselike substance. He regarded this mass as *eine wahre Balggeschwulst* (a true tumor of the skin or sebaceous cyst) which originated from the mucus-secreting structure of the inner surface of the gallbladder. Several later writers have referred to Wiedemann as the first to report an adenoma of the gallbladder. In all likelihood he described only an unusually large so-called cholesterol polyp. According to Weltz,[13] Meckel, in 1816, called attention to *Brüche* (breaks) in the mucosa of the gallbladder which, he thought, were comparable to those in the urinary bladder and the false diverticula of the intestinal tract. He found these most frequently at the base of the gallbladder and stated that such gallbladders usually contained gallstones. In fact, Meckel concluded that gallstones were concerned in their formation. So far as we are aware, this was the first time that the resemblance of these so-called glands to diverticula was mentioned.

Much of the subsequent literature on this subject was concerned with the question whether true glands were ever present in the wall of the gallbladder. For example, Cruveilhier,[14] in his "Anatomy of the Human Body," described minute features of the mucosa of the gallbladder and then announced: "After the most careful examination, I have been unable to recognize any crypts or follicles," [15] Huschke,[16] in 1844, found

10. Galéati, D. G.: [Mém. Acad. d. sc. de Bologne] Collect. acad. d. mém. **10**:416, 1773.

11. Morgagni, J. B.: The Seats and Causes of Diseases Investigated by Anatomy, translated from Latin by B. Alexander, London, A. Millar & T. Cadell, 1769, vol. 2, letter 37, pp. 212-279.

12. Wiedemann: J. d. pract. Arznk. u. Wundarznk. **3**:383, 1797.

13. Weltz, G. H.: Ueber Divertikel der Gallenblase, Kiel, L. Handorff, 1894.

14. Cruveilhier, J.: The Anatomy of the Human Body, New York, Harper & Brothers, 1844.

15. G. Cabibbe (Alti d. r. Accad. d. fisiocrit. de Siena **12**:437, 1900), however, stated that Cruveilhier was one of more than thirteen authors who "described real

(Footnotes continued on next page)

structures in the gallbladder representing what he called *aggregirten Drüsen* (compound glands), but Theile,[16] in 1845, while recognizing glands in the bile ducts (of which Luschka said he was the discoverer), could not find any in the gallbladder. Gubler,[17] in 1849, described a gallbladder with abnormal mucous follicles, especially at the fundus. Some of these contained bile-stained debris mixed with mucin and indicated to him that the gallbladder possessed mucous glands. Courvoisier,[18] in 1890, concluded that Gubler described the early stage of gallstone formation and noted that Verneuil[19] in 1854 had reported the observation of a gallbladder containing real stones, some free in the lumen and others located in the thickness of the mucosa. Wedl,[20] in 1850, after investigation, particularly of the glands of the bile ducts, could not find glands in the gallbladders of man, dogs or cows, and Kölliker,[21] in 1854, examined two human gallbladders and found no glands. He concluded that, though their occurrence in gallbladders had been reported, it was certainly not a constant finding. Gerlach,[16] in 1860, apparently observed glands in the gallbladder but stated that these "bile duct glands" were *spärlichsten* (most sparse) in the gallbladder and cystic duct. In 1851 Fauconneau-Dufresne,[22] in a comprehensive treatise on gallstones, stated that mucous crypts such as are present in the walls of the bile ducts are not found in the normal gallbladder, although between the folds of the mucosa are tiny spaces analogous to them. However, in the presence of gallstones glands are found. He mentioned that gallstones enclosed in glands had been described by Morgagni, Galéati and Mareschal. The last author, he said, had seen a gallbladder in which the dilated follicles formed tumors in the wall of the gallbladder.

In 1858 Luschka[23] published a study, *Die Drüsen der Gallenblase des Menschen* (The Glands of the Human Gallbladder), the first, to our knowledge, for which such a definite title had ever been employed and certainly the best exposition of these so-called glands which had been made up to that time. Luschka recognized the frequent disagreements in the literature as to the presence of glands, which he explained by the fact that the glands were irregularly distributed in the gallbladder, were usually small and, in many human gallbladders, numbered only three or four and hence were easily overlooked. Luschka stated that for a long time he had preserved beautiful examples of these glands in glycerin. By stripping off the mucosa, treating this with acetic acid and examining the transparent specimen against a glass plate held toward the light, he found in the cleared specimens flatly rounded or fine-streaked or aggregated nodules measuring up to 1 mm. in breadth and clearly demarcated from the surrounding tissue. They were present in those parts of the gallbladder which were covered by peritoneum as well as in those portions lying opposite, and also in the fundus. Their structure was acinous, *traubenförmige* (grapelike); rounded lobular groups with end bulbs were relatively rare. They were usually branched with irregularly sized oval *Ausbuchtungen* (outpouchings) which emptied into a common duct. This duct was occasionally long and winding and reached the mucosa in an oblique course. Rarely it possessed branches or simply outpouches so that the whole gland assumed a peculiar spread-out (*gespreizte*) appearance. Occasionally, clearly defined cysts were present. One specimen had five such cysts in the submucosa, and he had no doubt that these were derived from glands in which the ducts had been obliterated. There can be no question that Luschka observed and described the invaginations of epithelium into the wall of the gallbladder which later were frequently called *Luschkaschen Gänge* (Luschka's crypts) and that if any man's name should be attached to these structures, it should be his by reason of priority in identification and description of their chief features.

In his *Handbuch der speciellen pathologischen Anatomie,* published in 1842, Rokitansky[24] noted that small stones are occasionally located in *herniösen Ausbuchtungen* (hernia-like outpouchings) of the mucosa of the gallbladder, apparently completely separated from the lumen of the gallbladder. Later, in the *Lehrbuch der pathologischen Anatomie,* Rokitansky[25] slightly

glands of the mucous type in the mucosa of the gallbladder."

16. Cited by Luschka.[22]

17. Gubler: Compt. rend. Soc. de biol. **1**:24, 1849.

18. Courvoisier, L. G.: Casuistisch-statistische Beiträge zur Pathologie und Chirurgie der Gallenwege, Leipzig, F. C. W. Vogel, 1890.

19. Verneuil, cited by Courvoisier.[18]

20. Wedl, C.: Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl. **4** (pt. 2):480, 1850.

21. Kölliker, A.: Manual of Human Histology, London, Sydenham Society, 1854, vol. 2, pp. 122-223.

22. Fauconneau-Dufresne, V. A.: Traité de l'affection calculeuse du foie et du pancréas, Paris, V. Masson, 1851.

23. Luschka, H.: Ztschr. f. rat. Med. **4**:189, 1858.

24. von Rokitansky, C.: Handbuch der speciellen pathologischen Anatomie, Vienna, Braumüller & Seidel, 1842, vol. 2, p. 374.

25. von Rokitansky, C. F.: Lehrbuch der pathologischen Anatomie, Vienna, W. Braumüller, 1861, p. 282.

enlarged on this statement as follows: "There also occur in the gallbladder hernial outpouchings of the mucosa, at times, in large number, usually of small size (millet and hempseed). They have very narrow duct-openings so that the darkly colored concretions so commonly found in them appear to be located within the bladder wall." It is quite clear that it was the presence of the enclosed calculi that attracted the attention of Rokitansky, just as it had that of Morgagni and earlier writers, although it must be admitted that he recognized more clearly the circumstances of their formation. However, he gave no descriptive details and has little more claim than Galéati to their discovery.

In the second volume of his *Anatomie des Menschen,* published in the years from 1863 to 1869, Luschka [26] again considered glands of the gallbladder. He mentioned the blind, ductlike *Grübschen* (pits) in the common bile duct and stated that similar structures were found in the inner wall of the gallbladder, although, compared with the bile ducts, the gallbladder appeared relatively lacking in glands; yet he always found them present. He insisted that the true glands of the gallbladder must be differentiated from *Hohlgebilde* (spaces) in the wall, which usually contained particles of bile pigment and were found in that part of the wall covered by peritoneum. However, he emphasized that he had never found that these opened into any cavity and considered that they represented the metamorphosis of embryonal structures. His description of the glands as well as of these *Hohlgebilde* is not at all clear, and later writers, notably Halpert, concluded that he was describing, among other structures, blind remnants of bile ducts seen occasionally in that part of the gallbladder wall adjacent to the liver.

In spite of this already formidable array of facts, Henle,[27] in 1866, stated in his "Systematic Anatomy" that mucous glands are only rarely found in the gallbladder and that Theile, Wedl and Kölliker *vermissen sie gänzlich* (failed completely to find them). He, however, acknowledged that Luschka had described them.

In 1899 Hoppe-Seyler,[28] in a historical review of gallstones, asserted that Malpighi discovered glands in the gallbladder and the bile ducts. However, in 1903, in an article in the American edition of Nothnagel's Encyclopedia of Practical Medicine,[29] he said that medicine was indebted to Morgagni for this discovery and that Morgagni "further gives irritation of the glands of Malpighi in the wall of the gallbladder as another predisposing cause for gallstone formation." Exactly the opposite view was expressed in 1889 by Zenker,[30] who studied the relation of gallstones to carcinoma of the gallbladder. He concluded that gallstones caused ulceration and scar formations in the gallbladder and that these in turn led to increased growth of the mucous glands and, by gradual transformation of these glands, to carcinoma. He found that usually glands in the gallbladder were *ausserordentliche spärlich* (extraordinarily scarce) and he himself, in numerous preparations, could find only 2 which contained glands such as were described by Luschka and Gerlach.

A very thorough study of the pathology and surgery of the gallbladder was published in 1890 by the surgeon Courvoisier.[18] In 375 pages with more than 2,000 references, he covered historical aspects as well as the experiences of his own time. However, his references to glands in the gallbladder were only fragmentary. In a chapter entitled *Ein- und Abschnürungen* (loculation and constriction), *Divertikel der Gallenblase* he said that Heister (1717), Ruysch (1721) and Amyand (1738) had given the first descriptions of diverticula of the gallbladder and that Morgagni (Adversaria, 1747) likened these to similar structures in the urinary bladder and called them hernias. Similarly, he said, Meckel, in 1816, spoke of such diverticula completely separated from the lumen and frequently containing calculi. Courvoisier noted Rokitansky's reference to hernia-like mucosal invaginations, many of them only the size of a grain of millet. He concluded that these explained Morgagni's and others' observations of concretions in the gallbladder wall. He collected reports of 28 gallbladders which had what he called "special diverticula." In 11 instances the diverticulum was located in the fundus of the gallbladder, and in 18 of the 28 specimens there were calculi in the diverticula. He was uncertain whether stones preceded the diverticula or vice versa

26. von Luschka, H.: Die Anatomie des Menschen in Rücksicht auf die Bedürfnisse der praktischen Heilkunde, Tübingen, H. Laupp, 1863-1869, vol. 3, pp. 235-263.

27. Henle, J.: Handbuch der systematischen Anatomie des Menschen, Braunschweig, Friedrich Vieweg u. Sohn, 1866, vol. 2.

28. Hoppe-Seyler: Cholelithiasis, in Quincke, H., and Hoppe-Seyler, G.: Die Krankheiten der Leber, in Nothnagel, H.: Specielle Pathologie und Therapie, Vienna, Alfred Hölder, 1899, vol. 18, pt. 1, pp. 180-265.

29. Hoppe-Seyler: Cholelithiasis, in Nothnagel, H.: Encyclopedia of Practical Medicine: Diseases of the Liver, Pancreas and Suprarenal Capsules, translated by A. Stengel, Philadelphia, W. B. Saunders & Company, 1903, p. 525.

30. Zenker, H.: Deutsches Arch. f. klin. Med. **44:** 159, 1888-1889.

but was inclined toward the view that the diverticula were secondary to the calculi. When there was obstruction of the common duct he called them "pulsion diverticula." If there was adhesion to the colon there might be produced a "traction diverticulum." Quite evidently he made no distinction between true diverticulum of the gallbladder, which contains all the coats in a hernia-like outpouching and is a rare occurrence, and false diverticulum, in which the wall is invaginated only by the mucosal epithelium. In another chapter he considered benign tumors of the gallbladder and, with no cases of his own, collected only 3 from the literature. One was reported by Wiedemann, as we have already noted. A second was reported in 1862 by Albers, who described a submucosal sharply circumscribed "fibroid." The third was from von Schueppel [31] and was called a case of papillary myxoma. Not one of the three descriptions corresponds to later descriptions of so-called "adenomas."

In 1892 Adler [32] reported that in a man 37 years of age, cholecystectomy was deemed necessary because stones were present in the wall as well as in the lumen. He concluded that they were not in cysts because no epithelium surrounded them, although he did find cysts lined by epithelium and containing mucus and cholesterol debris. Adler related that Professor Waldeyer, who saw this specimen, remarked that it was more probable, though not proved, that these cysts represented a dilatation of the vasa aberrantia, first noted by Luschka as occurring frequently in the extrahepatic bile ducts and also in the left triangular ligament as well as, very rarely, in the wall of the gallbladder. Adler further observed in this specimen a calculus in the wall near the cystic duct entirely surrounded by normal mucosa. When called on by Israel [33] to discuss this complication as an indication for cholecystectomy, Langenbuch [34] said he had read of cysts in the gallbladder wall but had never seen them, though he had encountered gallstones embedded in the wall, which, he concluded, arrived there by an ulceration. He added that Thiriar, of Brussels, had had a similar case.

In 1892, in a famous thesis offered as proof that 90 per cent of all women who had gallstones had also borne children, Schroeder [35] mentioned that in a gallbladder which had been removed at a postmortem examination by Professor von Recklinghausen a calculus was found in a diverticulum-like pouch.

Weltz,[36] in 1894, employed, for the first time, the title *Ueber Divertikel der Gallenblase,* for his inaugural dissertation. He quoted both Rokitansky and Luschka (the latter from his work on anatomy) and concluded that they were referring to the same structures in the gallbladder. Weltz reported details of 5 cases in which the gallbladders contained spaces in the walls, some with tiny ducts leading to the mucosa and others without any opening. He said that Professor Heller had in his collection a gallbladder in the fundus of which was an *Ausbuchtung* that possessed a tiny passage, so that the enclosed thickened bile could be pressed out. As structures that Weltz called glands were often seen arising from the walls of these spaces, he concluded that the bile which they contained caused, by irritation, a new formation of glands. He found no explanation for the outpouchings and hence denominated them "congenital diverticula." One of his specimens showed glandular hyperplasia forming an adenoma, and he asserted that up to that time no adenoma of the gallbladder wall had been described. His final conclusion was that the diverticula might favor the formation of gallstones as well as of carcinoma.

The following year Müller [37] wrote his inaugural thesis on the pathologic significance of glands in the human gallbladder.[38] He noted that Zenker [30] had stated that the glands of the gallbladder are so sparsely present that some authors have denied their existence and that Zenker in examination of numerous specimens had found only 2 with glands. Zenker, he added, endeavored to explain the presence of an increased number of glands by the hypothesis that ulcerative processes had destroyed some and regeneration had reproduced many more. Müller examined 26 gallbladders. Twenty of these were normal, and in them he found no glands, although he admitted that they were probably present and that he was unlucky enough to miss them. In 5 gallbladders there were gallstones,

31. von Schueppel: The Diseases of the Biliary Passages and Portal Vein, in von Ziemssen, H.: Cyclopaedia of the Practice of Medicine, New York, William Wood & Company, 1880, vol. 9, p. 568.

32. Adler: Deutsche med. Wchnschr. **18**:57, 1892.

33. Israel: Deutsche med. Wchnschr. **18**:57, 1892.

34. Langenbuch: Deutsche med. Wchnschr. **18**:57, 1892.

35. Schroeder, H.: Beitrag zur Aetiologie und Statistik der Cholelithiasis, Strassburg, C. Göller, 1892.

36. Weltz, G. H.: Ueber Divertikel der Gallenblase, Kiel, L. Handorff, 1894.

37. Müller, C. A.: Zur pathologischen Bedeutung der Drüsen in der menschlichen Gallenblase, Kiel, L. Handorff, 1895.

38. This, like that of his predecessor Weltz, was from Kiel, Germany; both were sponsored by their professor, Heller.

and in all of these gallbladders he found numerous glands. He debated whether the gallstones by irritation had caused the few glands normally present to undergo a marked increase in number but finally was convinced that the glands were already abnormally numerous in these gallbladders and, because of the increased production of mucus, had caused the formation of gallstones. His one remaining specimen was a carcinoma of the gallbladder, which he concluded was the direct consequence of increase in glands. In 4 of the 5 specimens with gallstones he noted hypertrophy of the muscle bundles. These two dissertations illustrate the confused state of knowledge about gallbladder "glands," a confusion which has existed in a varying degree up to the present time.

The first notice of these structures in the United States was found in a book "On Gallstones or Cholelithiasis," by Brockbank,[39] published in 1896. He stated that, while most gallstones developed in the lumen of the gallbladder, he had observed 2 specimens with the mucosal surface dotted by tiny dark specks like grains of sand, which, with a sharp pointed knife, could be enucleated. They proved to be tiny gallstones that were contained in spaces of the mucosa, like retention cysts. He said he had described these extramucosal gallstones in the *Manchester Medical Chronicle* for December 1893 and January 1894. He then added that "large concretions also lie at times in a saccular outgrowth from the wall of the gallbladder," and that "in rare cases the gallbladder is found to be like a honey combed structure with no distinct central cavity." This, he concluded, was an exaggeration of the spaces in which the small black particles were found.

A third dissertation came from Freiburg, where Zenker's pupil, Schiff,[40] wrote, in 1898, on the glands of the gallbladder and their pathologic significance. Most of his paper was devoted to a historical review of the old question whether the gallbladder contains glands. He noted that Theile and Wedl could not identify them and that Kölliker, after Luschka's publication, grudgingly admitted their occurrence but insisted that they were not always present. But, according to Schiff, the majority of writers on anatomy and histology agreed more or less completely with Luschka's conclusions. Among the latter group, Schiff named Henle, Krause, Gengenbauer, Hyrtl, Bohn and Davidoff, Stohr and Rauber. He reviewed in detail the theses of Weltz and Müller. His own study was limited to 5 gallbladders, which contained gallstones. In 4 of these he found no glands (*Drüsen finden sich nirgends*). In the fifth specimen there were numerous glands lined by high cylindric epithelium. He concluded that these glands were not the result of gallstone or other irritations. He analyzed 8 other cases and tried to prove that in these the glands predisposed to the formation of carcinoma, a hypothesis to which Zenker had already been committed.

The following year Bolay,[41] in a thesis from Lausanne, Switzerland, discussed normal and pathologic glands of the gallbladder. He quoted from the "Anatomy" of Testut, published in 1895, to the effect that the gallbladder contains glands in variable number but that these were always more numerous near the neck, and commented: "For some histologists they are true mucous glands; for others, they are simply invaginations of the epithelial mucosa." Bolay reported observations on 29 normal gallbladders. In 18 he found tubular glands, the presence of which, he stated, was not disputed by any one. In 6 there were acinous glands, a type which was the basis of controversy. What he denominated as Luschka's glands (without describing them) he found in 3 gallbladders, but in 8 there were no glands of any kind. Because one of the gallbladders came from a child, 4 years of age, and contained a large number of acinous glands, he concluded that they had been preexistent and that their formation bore no relation to gallstones or irritation. However, he was willing to concede that these glands might predispose to cholelithiasis. In a study of 16 gallbladders containing calculi he found acinous glands in all but 1 and this had "Luschka's glands." He concluded that in gallbladders with stones acinous glands were always present, but he was unwilling to state definitely what was the relation, if any, of these glands to the gallstones. He emphasized that the acinous glands had no relation to the "tubes de Luschka," without giving any more detailed explanation of what he meant.

Cabibbe,[15] in 1900, studied gallbladders and common bile ducts from guinea pigs, rabbits and dogs. He constantly observed and, according to Shikinami, was the first to mention epithelial invaginations of the mucosa of the gall-

39. Brockbank, E. M.: On Gallstones or Cholelithiasis, Philadelphia, P. Blakiston, Son & Co., 1896.

40. Schiff, J.: Ueber die Drüsen in der Gallenblase und ihre pathologische Bedeutung bei Cholelithiasis und primärem Carcinom der Gallenblase, Freiburg i. B., E. Kuttruff, 1898.

41. Bolay, R.: Recherches sur les glandes de la vésicule biliaire et l'état normal pathologique, Lausanne, C. Pache-Varidel, 1899.

bladder but concluded that these should not be regarded as glands, because of the similarity of the epithelium lining them to that of the cavity to which they belonged, as well as because of the variability of their formation. He considered the invaginations to be primitive hepatic ducts. Sudler,[42] in 1901, likewise studied the structure of the gallbladder in animals and found tubular glands, a few in the dog, "but in the pig and, especially in the ox, they are quite numerous."

Bishop,[43] in 1901, reported "An undescribed innocent (?) growth of the gallbladder." In a woman aged 42 years he encountered a gallbladder which was the size of a child's head and was filled with cystic cavities containing bile and mucus and lined by a single layer of epithelium. There were no evidences of cancer in the specimen. He was probably unaware of the fact that Brockbank,[39] in 1896, had mentioned a somewhat similar case. In 1902 Durante [44] found in a gallbladder (containing gallstones) which he had removed numerous and extraordinarily developed glands. He thought that their presence tended to confirm Müller's hypothesis of the close relation of these glands to the formation of gallstones.

In an extensive review of what he called *Gallensteinkrankheit,* Törnqvist,[45] in 1903, discussed glands in the gallbladder. He stated that Soulie, in Poirrier and Charpey's *Traité d'anatomie humaine,* had described two types of these structures. One, located in the mucosa, had the form of a diverticulum, like the finger of a glove expanded in its deeper portion; the other, located in the middle coat, had the appearance of a true acinous gland. Törnqvist examined the fundal portions of 12 normal gallbladders. In none of these could glands be demonstrated, but in 2 specimens glandlike structures, superficially situated, were located in the mucosa. Of 33 specimens, excised because of gallstones, 20 contained glands. He concluded that calculus formation and glands were in a close causal relationship, the glands having been developed in consequence of the irritative effect of the stones. He quoted Radsiewsky,[46] who, in 1902, experimentally produced in dogs a fistula between the gallbladder and the duodenum and found in the gallbladder a notable increase of glands which had penetrated deeply into the wall between the smooth muscle bundles. Törnqvist also reviewed the literature concerned with intramural gallstones and noted that Courvoisier had cited Bianchi's discovery of stones in glands of the gallbladder. Similar observations, he noted, had been made by Morgagni and Fauconneau-Dufresne. He referred to Gubler's report of a gallbladder with abnormal mucous follicles and stated that, according to Hedenius, a similar instance had been mentioned by Santorini. Hedenius also, he said, found in the gallbladder of a woman 40 years of age glands developed in the wall and closed off from the lumen. The peritoneal covering of the gallbladder apparently had perforated, and light pressure caused several stones to escape into the peritoneal cavity. The lumen of this gallbladder contained 336 stones.

Mayo Robson,[47] in 1905, reported the discovery of an "adenoma" of the gallbladder which was taken from a woman 53 years of age. It consisted of "loculi" occupying the fundus and containing crystals of cholesterol. He referred to Bishop's report of a similar specimen and the 3 others described by Wiedemann, Sutherland and Terrier and Auvray.[48]

Among the large number of contributions written about the gallbladder and gallstones, the paper of Aschoff [49] on the pathologic anatomy of cholelithiasis and cholecystitis, delivered before the German Pathologic Society in 1905, stands out as a classic. He studied 145 gallbladders which had been sent to him by the surgeon Kehr. One portion of this study, of which he gave a comprehensive discussion, was concerned with what he called Luschka's *Gänge.* He began by noting the existing controversy over the presence or the absence of glands in the gallbladder and explained the discrepancy of past reports by the fact that some authors had examined normal gallbladders, in the fundus of which he, Aschoff, had completely failed to find true glands, while others were describing diseased gallbladders. However, Aschoff did note that in many gallbladders there were *Einsenkungen* (invaginations) of the surface epithelium along the path of the vessels passing between the muscle bundles. These pouches could travel clear through the muscle coat to the

42. Sudler, M. T.: Bull. Johns Hopkins Hosp. **12**: 126, 1901.

43. Bishop, E. S.: Lancet **2**:72, 1901.

44. Durante, F.: Bull. d. r. Accad. med. di Roma **28**:133, 1902.

45. Törnqvist, G. W.: Nord. med. Ark. (no. 1) **3**:1, 1903; (no. 7) **3**:97, 1903.

46. Radsiewsky, cited by Törnqvist.[45]

47. Mayo Robson, A. W.: Lancet **1**:999, 1905; Med.-Chir. Tr. **88**:229, 1905.

48. L. F. Terrier and M. Auvray (Chirugie du foie et des voies biliaires, Paris, F. Alcan, 1901) stated that benign tumors of the biliary system were rare. They mentioned the tumor described by Wiedemann and a few others which manifestly were not derived from glands. They reported no cases of their own.

49. Aschoff: Verhandl. d. deutsch. path. Gesellsch. **9**:41, 1905.

tunica fibrosa and represented the *Gänge* described by Luschka.[50] Aschoff thought these structures played an important role in the pathologic anatomy of cholelithiasis and cholecystitis inasmuch as gallbladders containing stones and changed by chronic inflammation showed Luschka's *Gänge* markedly increased in number as well as in the depth of their penetration through the muscle layer of the gallbladder wall. This point he had emphasized two years before in his report to the surgeon Kehr about gallbladders removed by operation. He said they were the glands described by Müller, Törnqvist and others. Stasis causing increased pressure within the lumen of the gallbladder, he concluded, changed the small crypts to "false diverticula." In extreme cases they could increase in size so that the entire gallbladder wall appeared like a sponge. Their relation to the clefts made by the blood vessels reminded him of the false diverticula of the colon. He stated that gallstones might be formed in these crypts and thus appear directly beneath the serosa, as Rokitansky had believed. Aschoff was inclined to the view that gallstones caused the crypts, by increasing the internal pressure in the gallbladder. This was indicated by hypertrophy of the smooth muscle which accompanied the dilated crypts. In the presence of chronic inflammation of the gallbladder, he hinted, there was active proliferation of glands. Infection of the *Gänge* might cause abscesses in the gallbladder wall, and these might later rupture into the peritoneal cavity.

In the following year Lubarsch,[51] before the German Pathologic Society, gave another slant to the structures that Aschoff had described at the preceding meeting. In 180 postmortem examinations of elderly people he had observed 9 gallbladders that presented a flattened thickening of the entire wall; when sectioned the wall had a spongelike appearance and on microscopic study revealed such a large number of dilated, cystlike glands in the muscle and subserous coats that one might suspect the presence of cystadenoma. The muscle bundles collected around them suggested even adenomyoma. He considered these to be "blastoma-like heterotopic growths" produced by inflammation. According to him, their basis was congenital heterotopia of the epithelium, and he thought they could precede the development of carcinoma, although he admitted he could discover no proof for this conclusion. Similar structures in the intestine, he concluded, were postembryonal. In this same year Pels-Leusden[52] asserted that Luschka's crypts, as well as papillomas of the gallbladder, were due to irritation resulting from calculi or from inflammation. Also in 1906, Otten,[53] a pupil of Aschoff, wrote on Luschka's crypts. He expressed doubt whether Luschka ever saw these structures and whether rightly they should bear his name.

Ehrhardt,[54] in 1907, noted Luschka's paper of 1858 and reviewed some of the later literature. He studied 6 normal gallbladders and concluded that the crypts described by Luschka were present in the fundus of every gallbladder as well as in other portions covered by peritoneum and those lying adjacent to the liver. He agreed completely with the contention of Aschoff inasmuch as in 40 gallbladders which contained gallstones he found increase in the number and the extent of these "glands." They infiltrated the wall, sometimes clear to the serosa, and he regarded them as new growths which resulted from chronic inflammation due to gallstones. He adopted without critical analysis Aschoff's conception of passive invaginations of the mucosa of the gallbladder, penetrating into the underlying structures, due to increased intracystic pressure, and also Aschoff's conception of active proliferation of new glands, as well as active increase in the extent of the crypts, due to chronic cholecystitis with lithiasis. The new glands were supposed to be a response to "the influence of the chronic inflammatory irritation." He noted the peculiar complex arrangement of the muscle bundles in the wall of the gallbladder with the intervening connective tissues in which the blood vessels penetrated to the mucosa. These intermuscular spaces afforded the mucosal epithelium the paths for invasion producing what might properly be called "false diverticula," like those of the colon. Neither he nor Aschoff noted the paradox of comparing the diverticula of the colon, which never revealed active gland formation, with diverticula of the gallbladder, which, they assumed, were often marked by new growths. Two of Ehrhardt's specimens had calculi in the crypts. He stated that true diverticula of the gallbladder were rare, as Courvoisier had found only 28 cases and he himself had seen only 2. He concluded that the crypts might account for the

50. Aschoff remarked that Luschka's description in his "Anatomy" was not entirely clear. It is not certain that he consulted Luschka's earlier paper on glands of the gallbladder.

51. Lubarsch: Verhandl. d. deutsch. path. Gesellsch. **10**:208, 1906.

52. Pels-Leusden, F.: Arch. f. klin. Chir. **80**:128, 1906.

53. Otten, C.: Beitr. z. klin. Chir. **48**:141, 1906.

54. Ehrhardt, O.: Verhandl. d. deutsch. Gesellsch. f. Chir. **36** (pt. 2):496, 1907.

recurrence of gallstones. At a later meeting of the German Pathologic Society, in 1907, Hirsch [55] commented that Luschka's crypts were a fruitful source of clumps of mucus and coagulated protein, thus favoring the production of gallstones.

In 1908 Shikinami [56] published a long review of the microscopic anatomy of the gallbladder. He discussed at length the question whether the gallbladder or its glands secrete mucus and thus was led to a consideration of Luschka's crypts. He thought Luschka's name should not be attached to them because they had been noted by many others long before Luschka, including Ruysch, Bianchi, Vicq d'Azyr, Haller and Gerlach. The only credit he was willing to accord to Luschka was that of being the first to illustrate the glands which he described. He, like Aschoff, did not mention Luschka's earlier paper of 1858 and, like Aschoff, he was puzzled by the statement in Luschka's "Anatomy" that no opening could be discovered for the spaces which he described and the further conclusion that they represented metamorphosed remnants of embryonic structures related to the development of the liver. Shikinami thought that Cabibbe was the first to speak of "epithelial invaginations of the gallbladder mucosa" but that if any name was to be attached to these structures it should be that of Aschoff. He emphasized that the epithelial cells of the crypts exactly resembled those of the mucosa and that they were not glands, although they secreted mucus and thus resembled glands. He denied that aberrant bile ducts, such as Luschka had depicted, were ever present in human gallbladders. The crypts, described by Aschoff, were often branched and might communicate with the lumen of the gallbladder by a much smaller opening.

Jurisch,[57] in 1909, concluded that almost every author who mentioned crypts in the gallbladder was writing about identical formations, but he was emphatic in stating that Aschoff was not correct in naming these "Luschka's crypts." Neither in his publication of 1858 nor in his "Anatomy" did Luschka, according to Jurisch, deal with the structures which Aschoff had portrayed.

In their monograph on gallstones, published in 1909, Aschoff and Bacmeister [58] placed repeated emphasis on Luschka's *Gänge* in diseased gallbladders and reiterated Aschoff's previous statements. Stones in the crypts were likened to fecaliths. The "buds" from the crypts were regarded as true gland formations, as appears from the following statement: [59] "In the wall of these Luschka's *Gänge* under pathologic conditions true mucous glands develop, so that the Luschka's crypt serves as a duct for these glands." Thus they reasoned that there was a passive increase of crypts from intracystic pressure and an active growth of true glands from chronic inflammation. They also noted adenomatous structures in the wall of the gallbladder, which they described as glandlike cystic formations. They compared these to the malformations seen at the tips of Meckel's diverticula and regarded them as congenital anomalies which possibly played a role in the formation of carcinoma of the gallbladder. These adenomas of the fundus were present in about 3 per cent of gallbladders examined by them, twice in gallbladders without any inflammatory changes, thus supporting their hypothesis that the adenomas were congenital.

Konjetzny,[60] in 1910, discussed benign tumors of the gallbladder in some detail. He noted Wiedemann's report, in 1797, of a cyst in the wall of the gallbladder. Konjetzny thought the specimen described by Adler in 1892 was a similar structure. He referred to Kitt, who in 1901, in simple hydrops of the gallbladders of cattle, had observed large (*kolossale*) retention cysts in the wall. Konjetzny also stated that Kaufmann regarded as cystadenomas most of the small cysts which he occasionally observed in the wall of the gallbladder and which were hemispherical or spherical formations visible even on the outer walls. The descriptions of similar cysts offered by Lubarsch, Bishop and Mayo Robson were mentioned, as well as that of Sutherland, who observed an adenomyoma.

In 1910 Lestchinski,[61] a student of Askanazy, at Geneva, Switzerland, selected for his work and thesis, *Du rôle des conduits de Luschka dans la pathologie de la cholélithiase*. He reviewed the works of Aschoff and Ehrhardt on these structures and reported the results of two postmortem examinations performed by Askanazy. In one the gallbladder had collections of bile pigment and cholesterol in its wall, and in the other there was peritonitis, which, he concluded, arose from perforation of a deeply penetrating *conduit de Luschka*.

55. Hirsch, C.: Verhandl. d. deutsch. path. Gesellsch. **11**:155, 1907.

56. Shikinami, J.: Anat. Hefte **36**:551, 1908.

57. Jurisch, A.: Anat. Hefte **39**:395, 1909.

58. Aschoff, L., and Bacmeister, A.: Die Cholelithiasis, Jena, Gustav Fischer, 1909.

59. Aschoff and Bacmeister,[58] p. 19.

60. Konjetzny, G. E.: Ergebn. d. allg. Path. u. path. Anat. **14** (pt. 2): 712, 1910.

61. Lestchinski, A.: Du rôle des conduits de Luschka dans la pathologie de la cholélithiase, Geneva, E. Chaulmontet, 1910.

In 1911 Licini [62] reported an instance of adenocarcinoma associated with multiple cysts in the wall of a gallbladder from a woman 57 years of age. He said that cystic degeneration of the gallbladder was a disease already well known and referred to the publications of Wiedemann, Aschoff and Konjetzny.

Holmes,[63] in 1911, remarked that the epithelial cells lining the gallbladder and cystic duct penetrate into the walls and ramify between the muscle bundles even as far as the subserosa. According to him, these are the crypts described by Luschka and are not glands. One is also indebted to Holmes for reproducing verbatim the report made by Vicq d'Azyr on "glands of the gallbladder."

In 1912, in their monograph on gallstone disease, Grube and Graff [64] stated that the peculiar epithelium-lined invaginations of the mucosa of the gallbladder, which pass between the muscle fibers and even extend to the tunica fibrosa, are known as Luschka's *Gänge*. They are distributed throughout the entire wall but are not numerous. Báron,[65] in 1912, concluded that the localization of diverticula of the gallbladder at the fundus was the consequence of mechanical conditions. He indicated that the thick balls of mucus often present in the depths of diverticula represented the preformed framework of gallstones. As to effects produced in gallbladders by inflammation, he concluded that "the most important, because the most frequent, change was the hypertrophy of Luschka's glands."

Aschoff furnished the details which Kehr [66] gave on Luschka's crypts in his *Praxis der Gallenwege-Chirurgie* (Practice of Surgery of the Bile Passages), published in 1913. Kehr quoted Aschoff's description and added that the crypts did not appear in the fetus but were present in variable numbers and forms in normal gallbladders of adults. Further, he stated that "under pathologic conditions true mucous glands develop in the walls of Luschka's crypts." These statements were accompanied by two beautiful colored plates, one picturing Luschka's crypts and the other a cross section of three gallstones embedded in the wall of the gallbladder.

In the same year, Lecène [67] reported on microscopic lesions of 28 gallbladders (with gallstones) removed by surgical operation. He noted that most anatomists agreed that the only true glands in the normal gallbladder were occasional small mucous glands located near the cystic duct and analogous to those in the common and hepatic ducts. He also noted that what appear to be glands are only invaginations of the mucosa extending into the deeper layers. When gallstones are present, the invaginations of mucosal epithelium are prominent and often penetrate deeply. These are the structures which, since Aschoff's paper, are called *canaux de Luschka* or Luschka's *Gänge*. However, after reading Luschka's account in his "Anatomy," Lecène found the description "incomprehensible" and the structures to be interpreted only as aberrant biliary canaliculi, often found in the connective tissue layer between the liver and the gallbladder. At any rate, he did not believe that the formations described by Luschka bore any relation to the deep epithelial ingrowths so frequently encountered in gallbladders with gallstones; hence Luschka's name should be dropped from association with these. Lecène called them diverticula which resembled those seen in the sigmoid colon. He admitted that the exact mechanism of their formation was obscure but stated that pathologic distention of the gallbladder would appear to be an essential factor. He emphasized their pathologic significance, because calculi were often found in them and serious infections might result.

In this same year Savy, Bonnet and Martin [68] wrote about benign tumors of the biliary tract. They emphasized the rarity of these tumors and indicated that cystic formation constituted the majority of them. Including their own and those reported by others, they found 28 examples. Some were in the common duct and others were polyps as well as cystic masses. They concluded that previous chronic irritation, usually from stones, was a predisposing factor.

An extensive discussion of "The Mucous Glands of the Gall-Bladder" was published by Else [69] in 1915. He distinguished two types of glands, both found only in the adult gallbladder. One type resembled those glands seen in large bile ducts and did not extend into the muscle. The other, when fully developed, he called "Luschka's glands" and used the term "Luschka's ducts" for the tubules. His descriptions of

62. Licini, C.: Beitr. z. klin. Chir. **76**:770, 1911.

63. Holmes, B.: Lancet-Clinic **106**:678, 1911.

64. Grube, K. A., and Graff, H.: Die Gallensteinkrankheit und ihre Behandlung, Jena, G. Fischer, 1912.

65. Báron, A.: Beitr. z. klin. Chir. **77**:447, 1912.

66. Kehr, H.: Die Praxis der Gallenwege-Chirurgie, Munich, J. F. Lehmann, 1913, vol. 1, p. 150.

67. Lecène, P.: Presse méd. **2**:994, 1913.

68. Savy, P.; Bonnet, P., and Martin, J. F.: Lyon chir. **9**:673, 1913.

69. Else, J. E.: Northwest Med. **7**:12, 1915.

the latter agreed with those of Aschoff. However, he rejected the usual hypotheses advanced to explain their origin, namely, those of distention, infection and misplaced embryonal rests. He substituted what he called "a latent power of growth," which might possibly be awakened by infection, by distention, by a calculus or even by destruction of liver cells. He regarded them as a form of true glands and expressed the belief that they were important clinically because of their tendency to become infected, giving rise to abscesses or phlegmon, and also to harbor infection, which might be responsible for later complications, sometimes many years after the initial invasion. He recognized their resemblance to diverticula and also their apparently close association with adenomas of the fundus of the gallbladder.

Herxheimer,[70] in 1921, studied gallbladders removed surgically because of chronic inflammation. An outstanding lesion was "the prominence and especially deep penetration of the glandlike so-called Luschka's crypts." In some specimens, the crypts extended even to the serosa, and portions were cystically dilated. Herxheimer mentioned Aschoff's hypothesis of increased pressure as a cause of these but expressed the opinion that the active proliferation of the epithelium into the deeper layers of the gallbladder was due to chronic inflammation and compared this overgrowth to the atypical epithelial proliferation which results from inflammation in other regions of the body. He observed changes in the cells of the invaginations which indicated to him beginning carcinoma.

In the second edition of his work on biliary lithiasis, published in 1922, Chauffard[71] stated that in the various types of cholecystitis the mucosa of the gallbladder produced Luschka's crypts. In the presence of gallstones these formed tubules with the aspect of glands, but they were really pseudoglands because there was no differentiation of the epithelium. He called them parietal diverticula and said they were found most frequently in the fundus of the gallbladder. They, as well as the surrounding muscle, were markedly hypertrophic and ramifying when cholecystitis was present.

Hansen,[72] in 1922, commented on "stones in the wall" in chronic cholecystitis. In 10 cases without stones in the gallbladder, he found in the wall of the gallbladder under the mucosa, deep in the crypts of Luschka, one to several calculi from the size of a grain of millet to that of a pea.

Auster and Crohn[73] in the same year insisted that the only secreting tissue in the gallbladder is the mucous glands of Luschka. In this same year Bodnár[74] described "narrow clefts or rounded spaces" in the gallbladder. He noted that Aschoff and Bacmeister had stated that these represented an increase and dilatation of Luschka's crypts and that from their walls true alveolar-tubular mucous glands were developed. Bodnár examined a gallbladder from a woman 69 years of age, which contained, besides two gallstones, numerous spaces of varying size, some beneath the mucosa and others deep in the muscular coat, reaching even to the serosa. By means of serial sections he could demonstrate communication of some of these spaces with the lumen of the gallbladder and likened them to the condition "cystitis cystica" in the urinary bladder. He found what he thought were real glands on the margins of these crypts and expressed the belief, as did Lubarsch, that they represented active proliferation of the epithelium, which might prove to be precancerous.

Policard,[75] in 1922, compared the pseudoglands or crypts in the gallbladder to the crypts of Lieberkühn in the small intestine and viewed them as organs of epithelial regeneration. He stated that cholesterol calculi could form in the depths of these epithelial diverticula, which were especially well developed when stones were present in the lumen of the gallbladder.

In another study of the extrahepatic biliary passages, published in 1924, Aschoff[76] reaffirmed his previous views concerning Luschka's crypts and stated that in the normal gallbladder there were *so gut wie keine Drüsen* (practically no glands). He emphasized that the gallbladder was a special portion of the anlage of the liver and that Luschka's crypts revealed traces of this specialized development. Abell,[77] in 1923, gave a brief review of papillomas and adenomas of the gallbladder. Among 288 specimens, removed surgically, he found 8 benign tumors, 5 adenomas and 3 papillary adenomas. In 7 of the gallbladders there were gallstones and invariably the changes produced by chronic inflammation were present.

In the following years Halpert published several papers on the normal human gallbladder

70. Herxheimer, G.: Beitr. z. path. Anat. u. z. allg. Path. **69**:143, 1921.

71. Chauffard, A. M. E.: Leçons sur la lithiase biliaire, ed. 2, Paris, Masson & Cie, 1922.

72. Hansen, S.: Ugesk. f. læger **84**:405, 1922.

73. Auster, L. S., and Crohn, B. B.: Am. J. M. Sc. **164**:345, 1922.

74. Bodnár, L.: Virchows Arch. f. path. Anat. **238**: 359, 1922.

75. Policard, M.: Lyon chir. **19**:799, 1922.

76. Aschoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, 1924, p. 182.

77. Abell, I.: Ann. Surg. **77**:276, 1923.

from the standpoint of histology. The first,[78] which appeared in 1924, was concerned largely with the function of the extrahepatic biliary system. Halpert quoted Aschoff and Bacmeister to the effect that the presence of dilated Luschka crypts was pathognomonic of stasis in the gallbladder. In the second article,[79] published in 1925, he categorically stated that the Luschka crypts described by Aschoff were not glands and that true glands occur in the gallbladder only near the cystic duct. In 1926[80] he described in more detail hernia-like outpouchings of the mucosa of the gallbladder, structures that he now called Rokitansky-Aschoff sinuses. He stated that their presence and number, as well as their shape, length, diameter and manner of penetration, depended (1) on the relative strength and the development of the individual muscle bundles and their relation to each other, (2) on the number and the size of the blood vessels passing through the intermuscular spaces and their method of penetration and (3) on the state of contraction of the gallbladder and any condition, especially pathologic, which influenced its contractility. He further asserted that the true Luschka ducts are epithelium-lined structures in the wall of the gallbladder, either that portion covered by peritoneum or that part adjacent to the liver. He asserted that they do not communicate with either bile ducts or the lumen of the gallbladder and that Luschka, who first described them in 1862, had believed them to be metamorphosed remnants of the liver anlage.

These conclusions were further developed in a fourth paper,[81] in two parts, published in 1927. Halpert studied, in all, more than 1,000 gallbladders removed at postmortem examinations. He entitled the second part[82] "The 'True Luschka Ducts' and the 'Rokitansky-Aschoff Sinuses' of the Human Gall-Bladder." He again asserted that Luschka did not see and did not describe the structures which Rokitansky was the first to mention and Aschoff the first to describe in terms of modern histology. He referred to Luschka's method of examining the gallbladder as "rather primitive." He said that Luschka's statement that he found glands in that part of the gallbladder covered by peritoneum had led Aschoff to believe that Luschka referred to the crypts noted by Rokitansky and himself and that Luschka had misinterpreted these as true glands. Halpert admitted the possibility of such a misinterpretation but thought that more probably Luschka was describing only the aberrant bile ducts which, Halpert asserted, contrary to Aschoff and others, are to be found in those portions of the gallbladder covered by peritoneum as well as those adjacent to the liver. Therefore, Halpert repeated, the structures which Rokitansky called hernia-like outpouchings of the mucosa of the gallbladder, which Aschoff described as "Luschka's ducts" and which Shikinami named "Aschoff's ducts," should be denominated "Rokitansky-Aschoff sinuses," as they were not at all identical with aberrant bile ducts, noted by Luschka. Halpert concluded from his study that in healthy gallbladders the Rokitansky-Aschoff sinuses "are rarely seen, and that if they were found, they were shallow and did not penetrate the muscular coats, which were dense enough to prevent deep outpouchings."

Higgins and Mann,[83] in 1926, and Higgins,[84] in 1927, in experiments on the gallbladders of guinea pigs, noted deviations from the usual orderly peristaltic waves of smooth muscles in other organs. In 1927, Higgins wrote of these as follows:

> . . . the vesicle did not empty by contraction waves. Rather there appeared to be independent areas of contraction. . . . Thus there is not a gradual even reduction in the extent of the tunic wall, but by the more or less independent contraction of these various areas, small herniations of varying size are produced irregularly over the surface of the vesicle. These small herniations, made possible by the peculiar disposition of the smooth-muscle bundles, usually appear first over the fundus and then gradually increase in number and extent. . . . As further reduction goes on and the capacity of the vesicle is greatly decreased, these mucosal folds become markedly increased. They branch frequently, anastomose with other folds and thereby form large irregular spaces in the lumen of the vesicle. Furthermore, large crypts arise within the elastic layer, formed by the outpocketings of the mucosa into the outer layers of the vesicle. These crypts, however, communicate by small openings with the gradually decreasing lumen of the gallbladder.

In 1926 Biancalana[85] reported the case of a woman 30 years of age whose gallbladder at postmortem examination contained a small adenoma in the fundus, which bulged somewhat toward the peritoneal surface. His illustration suggested a marked resemblance to the so-called bile duct adenoma of the liver.

78. Halpert, B.: Med. Klin. **20**:408, 1924.
79. Halpert, B.: Anat. Rec. **29**:359, 1925.
80. Halpert, B.: Anat. Rec. **32**:232, 1926.
81. Halpert, B.: Bull. Johns Hopkins Hosp. **40**:390, 1927.
82. Halpert, B.: Bull. Johns Hopkins Hosp. **41**:77, 1927.
83. Higgins, G. M., and Mann, F. C.: Am. J. Physiol. **78**:339, 1926.
84. Higgins, G. M.: Anat. Rec. **36**:129, 1927.
85. Biancalana, L.: Arch. ital. di chir. **16**:539, 1926.

Chiray and Pavel,[86] in the first edition of their book on the gallbladder, published in 1927, briefly mentioned structures which they called *canaux de Luschka* and which, they stated, were only deep grooves (*sillons*) in the mucosa. Under pathologic conditions these penetrated the wall even to the outer fibrous coat. They did not appear in the fetus but were observed constantly in the gallbladder of the normal adult, though often so limited in number that numerous microscopic sections might have to be examined in order to find them. These pseudoglands, they asserted, were lined by normal epithelium and differed from true glands, which were present only in the neck of the gallbladder, by their occurrence in all portions, notably the fundus. Important pathologic conditions might develop in them, and thereby their ducts might become closed, so that they were separated from the lumen. The second edition of the work of Chiray and Pavel,[87] published in 1936, offered no further data or opinions on this subject.

Genkin,[88] in 1927, reported the pathologic changes observed by him in 15 chronically diseased gallbladders in which no stones were present. He found in these gallbladders all the conditions which have been described in those with gallstones, including increase of mucous glands, which appeared even in the wall of the gallbladder. In spite of all the literature which dealt with so-called Luschka's crypts, neither these nor related formations were mentioned.

Nicod wrote a thesis in 1922 on adenomyoma of the fundus of the gallbladder and reported 4 instances of this condition. The thesis is not available to us but in a later paper,[89] published in 1927, he repeated his descriptions and noted the resemblance, on microscopic examination, to Luschka's crypts. He did not, however, suggest these crypts as an origin for the "adenomyomas" which he thought were "hamartomas" in the sense in which Albrecht used this term.

In their book on "Diseases of the Gall Bladder and Bile Ducts," published in 1928, Graham, Cole, Copher and Moore[90] stated that "the so-called Rokitansky-Aschoff sinuses, deeper outpouchings of the mucosa toward the external layers, have often been confused with these glands [described by Halpert], although the two have no relation whatever to one another." They quoted from Halpert's article the opinion that the sinuses "are almost always a manifestation of disease." They called attention to the rare condition in which bile is found in the peritoneal cavity without any evidence of perforation and indicated that some writers thought such a complication might follow the perforation of a hernial protrusion of the mucosa of the gallbladder through the wall, the so-called Luschka's canals. In this same year Weidlinger[91] gave details of what he called a "fibromyoadenoma" of the fundus of the gallbladder. His description differed in no essential detail from those of other "adenomas" or "adenomyomas" previously reported.

In 1929 Henke and Lubarsch published a handbook of special pathologic anatomy and histology. The chapter on the gallbladder and the bile ducts, written by Hanser,[92] gave a thorough coverage of many problems concerning diseases of these organs. Hanser devoted considerable space to Luschka's crypts, and the following sentences are abstracts or quotations of his comments. 1. The mucosa (of the gallbladder) has numerous folds produced by ingrowing and outgrowing proliferation. 2. Among these are "the so-called Luschka's *Gänge*, which are not glands but invaginations of the mucosa toward the serosa." 3. Only under pathologic conditions are true glands formed (in the gallbladder) except at the neck. 4. Luschka's crypts are a product of ectasia of age in the sense of pulsion diverticula. 5. Excessive growth (of Luschka's crypts), with forestages of epithelial buds, leads to so-called fundus adenomas. 6. The so-called fundus diverticulum occurs when Luschka's crypts increase to a large size in the fundus. 7. Luschka's crypts are surrounded with lymphocytes and plasma cells. 8. When there is an increase of these crypts the epithelium can undergo a marked transformation whereby the cells secrete mucin instead of pseudomucin. Furthermore, as Aschoff contended, true mucous glands, similar to those in the neck of the gallbladder, can bud off from the walls of the newly formed Luschka's crypts. 9. Some authors (Bodnár, 1922) have described unusual epithelial proliferation in deep-lying Luschka's crypts suggesting beginning carcinoma. 10. The presence of desquamated epi-

86. Chiray, M., and Pavel, I.: La vésicule biliare; anatomie - physiologie - sémiologie - pathologie - thérapeutique, Paris, Masson & Cie, 1927.

87. Chiray, M., and Pavel, I.: La vésicule biliaire et ses voies d'excrétion, ed. 2, Paris, Masson & Cie, 1936.

88. Genkin, I.: Arch. f. klin. Chir. **144**:752, 1927.

89. Nicod, J. L.: Ann. d'anat. path. **4**:133, 1927.

90. Graham, E. A.; Cole, W. H.; Copher, G. H., and Moore, S.: Diseases of the Gall Bladder and Bile Ducts, Philadelphia, Lea & Febiger, 1928, p. 165.

91. Weidlinger, E.: Arch. f. klin. Chir. **153**:180, 1928.

92. Hanser, R.: Gallenblase und Gallenwege, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 5, pt. 2, chap. 4, pp. 748-873.

thelial cells and other detritus in the spaces of Luschka's crypts suggested stasis and increased pressure in the gallbladder. 11. Calculi can occur in, and in the region of Luschka's crypts, as Naunyn and others have stated.

In 1929 Baumgartner [93] noted that MacCarty and Corkery [94] could point to but 1 adenoma after examining 5,000 gallbladders. He studied 4,575 gallbladders, removed by surgical operation, and found 24 adenomas, each located in the fundus, where, he stated, normally no glandular tissue is present. Their average size was 1 cm., the smallest being 4 mm. and the largest 3 cm. The average age of the patients was 46 years, the youngest being 26 years and the oldest 62 years. Only 6 of these gallbladders with adenomas contained calculi.

In 1930 Buzzi and Lascano Gonzalez [95] objected to the term "adenomyoma of the gallbladder," proposed by Nicod, because this led to confusion with other lesions of the female genital tract and also because these structures in the gallbladder were not adenomyomas but adenomas which were developed in the depths of the wall of the gallbladder. Their specimen was obtained by a surgical operation on a woman 42 years of age. The nodule in the fundus was 5 to 6 mm. in diameter and consisted of small vesicles filled with mucoid fluid. They favored the hypothesis of chronic irritation as the best explanation for these growths.

In 1930 Gosset, Duval, Bertrand and Moutier [96] published a paper on intramural calculi of the gallbladder. They concluded that these were important, because they often escaped diagnosis by palpation and did not give the classic signs and symptoms of gallstone disease. They stated without qualification that "interstitial lithiasis is always developed in the interior of the diverticula of Luschka." The illustrations which accompany their article are particularly beautiful and exact.

In 1931 King and MacCallum [97] yielded to an impulse, common among medical writers, and coined a new term, "cholecystitis glandularis proliferans (cystica)." They thought this was the most suitable designation for circumscribed or diffuse thickening of the wall of a chronically inflamed gallbladder, characterized by excessive epithelial proliferation and invasion of the wall, forming crypts, some of which became dilated to form cysts. From the literature they collected other names for this condition, such as "adenoma" (Ewing and Vallée), "polyp" (Sommer), "cystadenoma" (Lasnier and Estevan), "adenomatous polyp" (Veschi), "papilloma" (Hrnska, Pels-Leusden), "fibro-adenoma" (Ikeda), "fibromyo-adenoma" (Weidlinger), "carcinoma" (Slade), "cholesterol cysts" (Ewing, Kaufmann, and Rolleston), "cholecystitis cystica" (Bodnár), "precancerous proliferation" (Zenker) and structure "of no special significance" (Janowski). They studied 400 gallbladders removed by operation and found in 38 (9.5 per cent) the changes which they were describing. Twenty-five of these gallbladders were selected for special examination. In most instances the lesions were located in the fundus of the gallbladder, although in some they involved a greater area—in 1 case, as much as three fourths of the organ. Cross sections gave the appearance of numerous "glands," but there were no signs of neoplasia. Serial sections revealed that the deeper "glands" were continuous with the surface epithelium and apparently developed from it. They concluded that "the proliferative epithelial changes in the gallbladder should be regarded as the reaction of the epithelial cells to the chemical stimuli occurring in an inflamed gall bladder." The paper is chiefly remarkable because of the omission of any reference to Luschka's or Aschoff's crypts.

In 1931 Nuboer [98] indicated that he thought the dilatation of Luschka's crypts was produced by increased pressure within the gallbladder. In 1933, in a discussion of biliary dyskinesia, Newman [99] thought "spastic distension" of the gallbladder was probably accompanied by hypertrophy of the muscle of that organ and deepening of Luschka's crypts, thus also emphasizing the factor of pressure. In this same year, Wiglesworth [100] described a gallbladder, removed by a surgical operation, in which cystic glands had penetrated the abdominal wall. He concluded that the histologic picture differed in no respect from the one described by King and MacCallum.[97]

In 1934 Wellbrock [101] reported a study of adenomas of the gallbladder. In 9,550 gallbladders,

93. Baumgartner, C. J.: Surg., Gynec. & Obst. **49**: 780, 1929.

94. MacCarty, W. C., and Corkery, J. R.: Am. J. M. Sc. **159**:646, 1920.

95. Buzzi, A., and Lascano Gonzalez, J. M.: Ann. d'anat. path. **7**:943, 1930.

96. Gosset, A.; Duval, P.; Bertrand, J., and Moutier, F.: Presse méd. **1**:161, 1930.

97. King, E. S. J., and MacCallum, P.: Brit. J. Surg. **19**:310, 1931.

98. Nuboer, J. F.: Frankfurt. Ztschr. f. Path. **41**:198 and 454, 1931.

99. Newman, C.: Lancet **1**:785, 841 and 896, 1933.

100. Wiglesworth, F. W.: Canad. M. A. J. **29**:410, 1933.

101. Wellbrock, W. L. A.: Am. J. Surg. **23**:358, 1934.

removed surgically, he found 69 adenomas. All but 4 of these were in the fundus. Two were carcinomas. Forty-seven were in women and 22 in men. Thirty-eight were accompanied by stones, but the remaining 31 had no stones. In 1 gallbladder there were 3 small tumors. No mention was made of Luschka's (or Aschoff's) crypts.

Boyd,[102] in his "Surgical Pathology," published in 1942, stated that:

> . . . as a result of inflammation, possibly because of increased intracystic pressure, there may be outpouchings of the mucosa into or through the muscularis. These are known as Rokitansky-Aschoff sinuses. When the section is cut obliquely, so that the connection with the mucosal surface is not apparent, they may be mistaken for carcinoma. Sometimes there is apparently a proliferation of epithelium and a formation of new glands, a condition known as *cholecystitis glandularis proliferans*. A mass may be formed on the serous surface, or the proliferation may take place on the surface of the mucosa, forming a papillary projection or papilloma.

In Bailey's "Text-Book of Histology," published in 1940, Smith [103] described Rokitansky-Aschoff sinuses as "diverticula of the mucosa extending into the muscular and perimuscular layers." He expressed the belief that they indicated a pathologic change following prolonged overdistention of the gallbladder. Luschka's ducts, on the other hand, were ductlike structures lined with epithelium having no connection with the lumen of the gallbladder and to be interpreted as aberrant embryonic bile ducts.

In 1941 Thorsness [104] considered "The Relationship of 'True Luschka Ducts,' Adenomas and Aberrant Liver Tissue in the Wall of the Human Gall Bladder." He listed the hypotheses concerning adenomas of the gallbladder. They were benign neoplasms (Abel), diverticula of the wall due to the fundus being the weakest portion and to subsequent migration of the cellular elements (Wellbrock), Rokitansky-Aschoff sinuses (Wiglesworth), Luschka's ducts (Simon), cystitis cholecystica (Bodnár), fetal rests and cholecystitis glandularis by a process of metaplasia (King and MacCallum). Thorsness followed Halpert's interpretation of Luschka's ducts. He reported a mass in a gallbladder which contained sympathetic nerve fibers, a nodule of aberrant liver tissue and a benign adenocystic structure which, studied by serial sections, did not communicate with the lumen of the gallbladder.

Finally, in 1942 Lichtman,[105] in a book entitled "Diseases of the Liver, Gallbladder and Bile Ducts," repeated the conclusions of Halpert concerning the "true ducts of Luschka" and the Rokitansky-Aschoff sinuses. The latter, Lichtman asserted, may play a clinical role in inflammatory and in noninflammatory gallstone disease by becoming dilated and retaining bacteria and debris. He then identified the aberrant bile ducts with vasa aberrantia, which he said were well known to Ferrin in 1773 and were further studied by Weber (1842), by Jurison (1909) and by Halpert.

THE ANATOMY OF NORMAL HUMAN GALLBLADDER

No useful purpose would be served by describing the minute anatomic details, gross or microscopic, of the gallbladder. Entirely adequate descriptions have been given on many occasions by various writers, notably Aschoff, in 1905, and others. However, in connection with the subject of this paper, a few points deserve emphasis. Although the gallbladder is derived, embryologically from the foregut and many authors have noted the anatomic resemblance of the mature gallbladder to the intestine, there are several important features in which it radically differs from any part of the gastrointestinal tract. For one thing, the mucosa of the gallbladder is manifestly sui generis. It is thrown into delicate tufts or folds dividing the surface into a network of small polygonal spaces. Viewed through a microscope, these folds give the appearance of villi, irregularly developed and distributed. Boyd, who has a happy facility for word painting, described these structures as follows: "When the fresh gallbladder, immersed in water, is observed by reflected light the picture is a remarkable and beautiful one. Tall graceful folds and membranes, gossamer-like in their delicacy, can be seen floating in the fluid like sea-weeds in a marine pool."

Perhaps the most important fact is that the mucous membrane of the gallbladder has no muscularis mucosae. Also there are normally no glands in the gallbladder wall except some small groups of tubular, and occasionally racemose, glands near the neck, similar to those found in the extrahepatic bile ducts. However, the epithelial cells of the mucosa bear strong resemblances to the glandular epithelium which lines

102. Boyd, W.: Surgical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1942, pp. 315 and 321-322.

103. Smith, P. E., and others: Bailey's Text-Book of Histology, ed. 10, Baltimore, Williams & Wilkins Company, 1940, p. 493.

104. Thorsness, E. T.: Am. J. Clin. Path. **11**:878, 1941.

105. Lichtman, S. S.: Diseases of the Liver, Gallbladder and Bile Ducts, Philadelphia, Lea & Febiger, 1942.

the intestinal tract throughout. The vasa aberrantia are entirely absent in the gallbladder; if they are ever sufficiently identified as present, they may be regarded as curiosities of embryologic development. So-called aberrant bile duct remnants are fairly frequently to be demonstrated in that part of the wall of the gallbladder adjacent to the liver. Halpert contended that they are to be found in those portions covered by peritoneum, but we have wholly failed to confirm this observation.

Finally, the so-called muscular coat of the gallbladder is a unique structure. The only similar formation which remotely resembles it in complexity of arrangement is that of the muscle bundles of the urinary bladder. Numerous attempts have been made to present an orderly description of the confusing assembly of smooth muscle fasciculi of the gallbladder wall. Longitudinal, oblique and circular layers have been identified, but so many variations in arrangement have been found that a coordinated picture applicable to all gallbladders does not seem possible. There can be no doubt concerning the presence of these muscle bands, and no matter how furiously the battle rages as to their physiologic significance for the emptying of the gallbladder, they surely can undergo hypertrophy or atrophy similar to that of smooth muscle collections in other parts of the body. If hypertrophy is present, it indicates a definite degree of increased function of contraction and hence, in all probability, increase in intracystic pressure. Particularly significant are the unique observations of Higgins [84] and of Higgins and Mann [83] of the contractions of the muscle bundles in the walls of the gallbladders of guinea pigs. These contractions were not in the form of peristaltic waves but were isolated and at first independent. They produced small herniations "made possible by the peculiar disposition of the smooth muscle bundles." Thus was produced for the first time an ocular demonstration of the mechanism which normally contributes to temporary herniations of the mucosa and which under abnormal conditions might reasonably be expected to produce more or less permanent invaginations of varying degrees. To the factor of intracystic pressure must be added the specialized arrangement of the muscles of the gallbladder wall and their tendency toward unsynchronized contractions and relaxations. Between these irregularly arranged muscle bundles in the connective tissue spaces, blood and lymph channels extend up to the mucosa and thus furnish, at least theoretically, paths for the invagination of the overlying epithelial layer.

RESULTS OF THE EXAMINATION OF 495 GALLBLADDERS

The gallbladders that we studied were from two sources—postmortem examinations (175) and surgical operations (320). After a gallbladder had been carefully inspected with the aid of the dissecting microscope, specimens for microscopic study were removed from various portions of the wall, including that adjacent to the liver, that of the fundus and those of parts covered by peritoneum. Our special endeavor was to identify crypts in various normal and pathologic gallbladders. Naturally, the entire wall could not be studied by serial sections, and therefore in some gallbladders in which no diverticula were found, more extensive investigation might have revealed eventually an occasional herniation of the mucosa. However, sufficient portions were examined from each gallbladder to afford a fairly accurate picture of the relative number and depth of the crypts that were actually present. Those microscopic preparations that revealed diverticula were classified in three grades: grade 1, those in which the crypts extended to the muscular coat; grade 2, those in which crypts were observed in the intermuscular lacunae, and grade 3, those in which crypts extended through the muscle into the underlying connective tissues, occasionally reaching the serosa. The results of this analysis may be summarized as follows:

Among the 175 gallbladders from postmortem examinations, 112 were judged to be grossly and microscopically normal.[106] Sixty-five of these (58 per cent) apparently contained no diverticula. Thirty-eight of the remaining 47 revealed diverticula of grade 1, and only 9 disclosed diverticula that could be graded 2 or 3. The conclusion appears justifiable that anatomically normal gallbladders contain few diverticula, and these tend to remain more superficially located.

Of 33 gallbladders with evidences of previous inflammation (or so-called chronic cholecystitis) and without gallstones, only 4 (12 per cent) were free from diverticula, 6 (18 per cent) had diverticula of grade 1, while 23 (70 per cent) had diverticula graded 2 or 3. Another group was

106. The term "normal" admittedly has no exact significance. With strict anatomic interpretation it is doubtful whether any organ of the adult human body can ever be pronounced absolutely normal. As the term is used by us, it implies the absence of identifiable signs of any pathologic condition that could influence the functions of the gallbladder materially or, what is more important, could contribute clinical phenomena of disease. Manifestly, the disorders that are neurogenic cannot, as a rule, be measured by any anatomic criteria now available.

composed of 22 gallbladders with chronic cholecystitis but containing gallstones. Of these, diverticula were lacking in 8 (36 per cent), diverticula of grade 1 were present in 8 and diverticula of grades 2 or 3 were shown in 6. There were also 7 cases of cholesterosis and 1 case of acute cholecystitis with stones. The foregoing figures agree with those of Aschoff, Ehrhardt and others, who asserted that there is an increase in both the number and the depth of Luschka's crypts in gallbladders in which chronic inflammation has been present. The additional presence of gallstones does not apparently play any considerable role. Indeed, inasmuch as gallstones often are found in otherwise normal gallbladders, especially at postmortem examinations, diverticula theoretically should be fewer and less invasive. Our results tend to confirm this view.

The 320 gallbladders that were removed by surgical operation were from patients who presumably had definite signs or symptoms of cholecystitis. At any rate, the proportion of these gallbladders that contained no diverticula was distinctly less than that of normal gallbladders removed at postmortem examinations. For example, among 21 specimens which were considered by the surgical pathologist to show acute cholecystitis there were only 5 in which no diverticula were found; of 45 with a diagnosis of subacute cholecystitis, only 5 were without diverticula; of 78 with a diagnosis of chronic cholecystitis, only 8; of 72 with a diagnosis of chronic catarrhal cholecystitis, none; of 65 with a diagnosis of chronic cholecystitis with stones, only 3. Among the whole 320 gallbladders only 23 (7 per cent) apparently were free from crypts. Also, when present, the crypts were definitely increased in depth and number.

Analysis according to sex revealed that of the 320 surgical specimens, 238 were from female patients, while only 82 were from males. This proportion of almost 3 to 1 is illustrative of the well known preponderance of cholelithiasis and related complications among women. In regard to diverticula of the gallbladders of these patients, the two sexes were about equally affected. In the postmorten series, 52 per cent of the males and 53 per cent of the females had crypts in the walls of their gallbladders. Evidently sex does not play any role in the formation of diverticula in gallbladders.

As to age, among the 46 patients with normal gallbladders and diverticula in the series of postmortem examinations, 24 per cent were less than 30 years of age, whereas 76 per cent were 30 or more. In the surgical series the age incidence was more difficult to evaluate inasmuch as most of the gallbladders were seriously diseased. But in general there tended to be a definite increase in diverticula with advance in years.

A few special features observed during this survey are noteworthy. Three gallbladders contained structures commonly identified as adenoma. In 32 gallbladders there were formations resembling mucous glands which were closely associated with the diverticula. In the wall of 1 gallbladder there were embedded numerous calculi, manifestly incarcerated in diverticular pouches which occasionally contained also precipitated cholesterol and pigment granules. The relative thickness of the walls apparently bore no relation to the presence or the absence of diverticula. In some of the thickest specimens none could be identified, while in some of the thinner ones diverticula might be abundant and deeply penetrating. Only 1 specimen contained an intramural abscess. The epithelial cells that lined the diverticulum were in every instance exactly similar to those that lined the lumen of the gallbladder. No attempt was made to determine accurately a ductal opening for the diverticulum but the presence of dilated pouches and even actual cyst formation strongly suggested that the paths of discharge of some of the diverticula or at least some of their branches had been either partially or wholly obstructed. This was particularly true of the so-called adenomas. Likewise, attention was not given to the question of hypertrophy or atrophy of muscle bundles. Such a research might give valuable data, but the notable variations in smooth muscle produced over comparatively short periods render exceedingly difficult a correct interpretation of the status of the muscle at the time the diverticula were formed.

COMMENTARY

This review of the data furnished by the work of others and by our own investigations gives a perspective which appears to us to present a logical solution of the several problems with which the literature has been engaged for the past three hundred years or more. Perhaps the least important of these problems is the question of a proper term for the condition. The tendency to attach personal names to all publicized discoveries, whether original or not, whether the subject discovered is a mountain peak or a chemical element, is almost universal and is readily understandable. However, this custom has led to an untold number of controversies and often, in the bitterness and confusion that have been engendered, the essential significance of the sub-

ject under discussion is wholly missed or disregarded. The comparatively trivial fact of who discovered the North Pole becomes more important than the geographic meaning of the Pole itself. Thus the question as to whether Luschka's name or Rokitansky's or Aschoff's or the names of other investigators should be attached to these peculiar structures in the wall of the gallbladder has occupied an amount of attention altogether disproportionate to that which it deserves.

The facts are clearly evident. Many writers, even more than are cited in our review, unquestionably observed and more or less accurately described diverticular pouches in the gallbladder. Luschka was probably the first to give them anything like an adequate description, though he almost completely failed to evaluate their significance. His publication, in 1858, of an article on the glands of the gallbladder emphasized their frequency, their presence in that part of the gallbladder covered by peritoneum and their occasionally cystic character. In spite of Halpert's objections, there can be little doubt that Luschka actually saw and imperfectly described the structures which so long have borne his name. The idea that he was concerned only with aberrant or vestigial bile ducts in the gallbladder wall, which are almost entirely, if not completely, confined to that portion adjacent to the liver, is not based on sound premises. It is doubtful that Luschka ever saw such aberrant bile ducts, much more that he could ascribe to them the anatomic characteristics that he observed, for they rarely, if ever, possess such noteworthy deviations from their original vestigial form. They are embryonic remnants which undergo regression, not development.

The crux of objections to the use of Luschka's name arises from the descriptions given in his "Anatomy," which was published at least five years after his article on glands of the gallbladder. Almost all subsequent writers, including Aschoff and Halpert, have been confused by Luschka's wholly inadequate and careless description of structures of which, in his earlier work, he had given a fairly clear exposition. Especially his statement that these so-called glands never communicate with the lumen of the gallbladder became a stumbling block as to what he really was describing. The supposition that that portion of the "Anatomy" was written years before and never revised to conform with his paper of 1858 is perfectly logical. Furthermore, the diverticula which he, as well as Rokitansky and many others before and since his time, must have observed are at first glance confined to the inner lavers of the gallbladder. Their ducts are often tortuous and narrow and their ostiums completely hidden or even entirely absent. Without careful study of serial sections it is frequently impossible to identify the deeper crypts' openings into the lumen of the gallbladder, and it is no wonder that Luschka with the imperfect technic which was afforded to earlier students of microscopic anatomy was unable to envisage the more obscure details of the conditions which he observed.

The fundamental error committed by Luschka, as well as by most of the writers before and many since his time, was to regard these structures as glands. Only a few earlier observers, notably Haller, Meckel and Rokitansky, appear to have recognized the resemblance of the crypts to diverticula either of the urinary bladder or of the intestine. Even Aschoff, in spite of his exact knowledge about Luschka's *Gänge,* concluded that real mucous glands were occasionally developed from their walls.

There is abundant confirmation of the simple fact that the mucous membrane of the gallbladder, which is not supported by any limiting membrane corresponding to the muscularis mucosae of the intestine and which lies adjacent to loosely arranged and often widely separated bundles of smooth muscle, can easily be pressed into the intervening connective tissue spaces, especially along the paths produced by the channels of the lymph and the blood vessels. The word "pressed" is used advisedly. Although any resistance that might be offered by the various components of the gallbladder wall to invasion by the mucosa might be and undoubtedly is reduced to varying degrees at times, the initial impetus to this penetration must arise from an increase of intracystic pressure. Such an increase may not exceed to any extent the normal upward range of this pressure, which is affected both by the muscular contractility of the gallbladder and by the numerous factors which may, temporarily at least, obstruct the outflow of bile from the organ. Theoretically, the ideal setup for such a state would be a gallbladder with normal or, better, hypertrophied muscle in its wall and neurogenic stimuli which produce spasmodic closure of the sphincter at the terminal portion of the common duct and, at the same time, strong contractions of the smooth muscle constituents of the gallbladder wall. In 1923, Westphal [107] demonstrated experimentally such a pathologic-physiologic complex, and later writers, particularly by the use of special dyes and roentgen rays, have apparently confirmed its presence in man. The term

107. Westphal, K.: Ztschr. f. klin. Med. **96**:95, 1923.

"dyskinesia" is frequently employed to designate this condition which offers, in fact, about the only possible explanation for the fairly frequent observation of biliary colic without the presence of gallstones or any other logical causative factor.

According to our own observations and those of many others, the indentations of the mucosal epithelium may range from shallow grooves or pouches, which hardly merit the qualifying word "pathologic," to deeply penetrating, complexly branching invaginations. The latter structures may be partially or completely cut off from the surface and become dilated in whole or in part into cysts. They may appear in an isolated portion of the gallbladder, particularly the fundus, and thus assume some of the characteristics of the so-called adenoma, or they may involve the entire wall of the gallbladder or large portions of it. Typical gallstones may be observed in the crypts, some completely closed off from the lumen of the gallbladder and others in spaces with ducts through which the calculi may be expressed into the lumen. Occasionally, bile pigments and irregularly arranged collections of cholesterol crystals may be observed in the crypts, but as a rule these contain a transparent mucin-like fluid. Although the presence of bile pigments, cholesterol or biliary calculi indicates an additional pathologic factor in the diverticular pouches, there is no reason to believe that gallstones are pressed into them. Whenever bile can displace the normal secretions of the lining cells, precipitation of elements of the bile even to the formation of true gallstones may take place under the same conditions that govern these processes in the cavity of the gallbladder. All degrees of inflammation leading to abscess formation, complete obliteration of the epithelial cells that normally line the spaces or even rupture into the peritoneal cavity or the liver may and do occasionally occur. However, as a rule anatomic signs of inflammation are entirely absent, and there is little, if any, reason to conclude that infection or irritation plays other than a secondary role.

The question whether the epithelium of the gallbladder undergoes active proliferation has unnecessarily, it seems to us, bothered many investigators. Whenever for any reason there is undue dilatation or expansion of an epithelium-lined space, epithelial proliferation accompanies such expansion pari passu, unless this proliferation is inhibited by some pathologic condition. This is the rule in all epithelium-lined cysts, as well as in diverticular pouches throughout the body, and there is no more reason to regard it as a neoplastic process when it occurs in the gallbladder than there is to consider the epithelial lining of diverticula of the colon and other hollow viscera as examples of neoplasia.

The similarity of the crypts of the gallbladder wall to diverticula of the small intestine, the colon or the urinary bladder, as previously noted, has attracted earlier writers. They are all invaginations of the mucosa of hollow viscera which are supported by more or less irregularly arranged bundles of smooth muscle in the walls. They are not embryologically derived but acquired and therefore are properly called false or, better, acquired diverticula. Although the argument that they occur because of some genetic or inherited weakness of the walls cannot be disproved, the evidence is all in favor of the hypothesis that the "weakness" is one of natural structure and that the invagination of the mucosal epithelium follows abnormal pressures which are exerted during stages of abnormal contractions or relaxations of the underlying muscle bands. This view is supported by the fact that diverticula, particularly those in the colon, the urinary bladder and the gallbladder, are found in increasing numbers as age advances. Furthermore, in these organs the invaginations are more frequently observed where excess pressures and stasis are most likely to occur; for example, in the sigmoid flexure of the colon and the fundus of the gallbladder.

The prominent branching of diverticula of the gallbladder and their almost unlimited spread in the wall have undoubtedly led most writers to establish for such formations a special category and therefore a special etiologic and developmental basis. We are perfectly willing to admit that as diverticula they are sui generis and have no counterpart, in all their features, in any other diverticula of the human body, but we insist that the differences are to be explained rather by the peculiar construction of the gallbladder wall than by any other specialized mechanism.

While the initial invagination of the mucosa of the gallbladder into the underlying layers is unquestionably produced by increased intracystic pressure, perhaps with relaxation of the elements which compose the walls, further development of the diverticula, particularly budding of pouches and cyst formation, may be aided by the pressure of contained secretions. The elongated, narrow and usually tortuous ducts readily account for obstruction and stasis in the deeper crypts, and complete isolation from the lumen of the gallbladder may and frequently does occur by obliteration of the excretory passages. The exact mechanism of this obliteration is not clear, but there is little evidence in favor of the hypothesis

that inflammation is the usual exciting cause. When the main duct is cut off, an isolated intramural collection of spaces may occur, and thus the so-called adenoma is produced. If only certain branches are obstructed, single or multiple cystic formations result. It is probably true that complete occlusion of ducts is more rare than is generally believed. Careful study of serial sections will usually reveal openings, even though tiny and narrow, into the lumen of the gallbladder.

Almost all investigators are agreed that there is an astonishing lack of evidence of neoplastic activities on the part of the epithelial cells which line the crypts. Our own studies confirm this fact. Hence the view that carcinoma is the natural

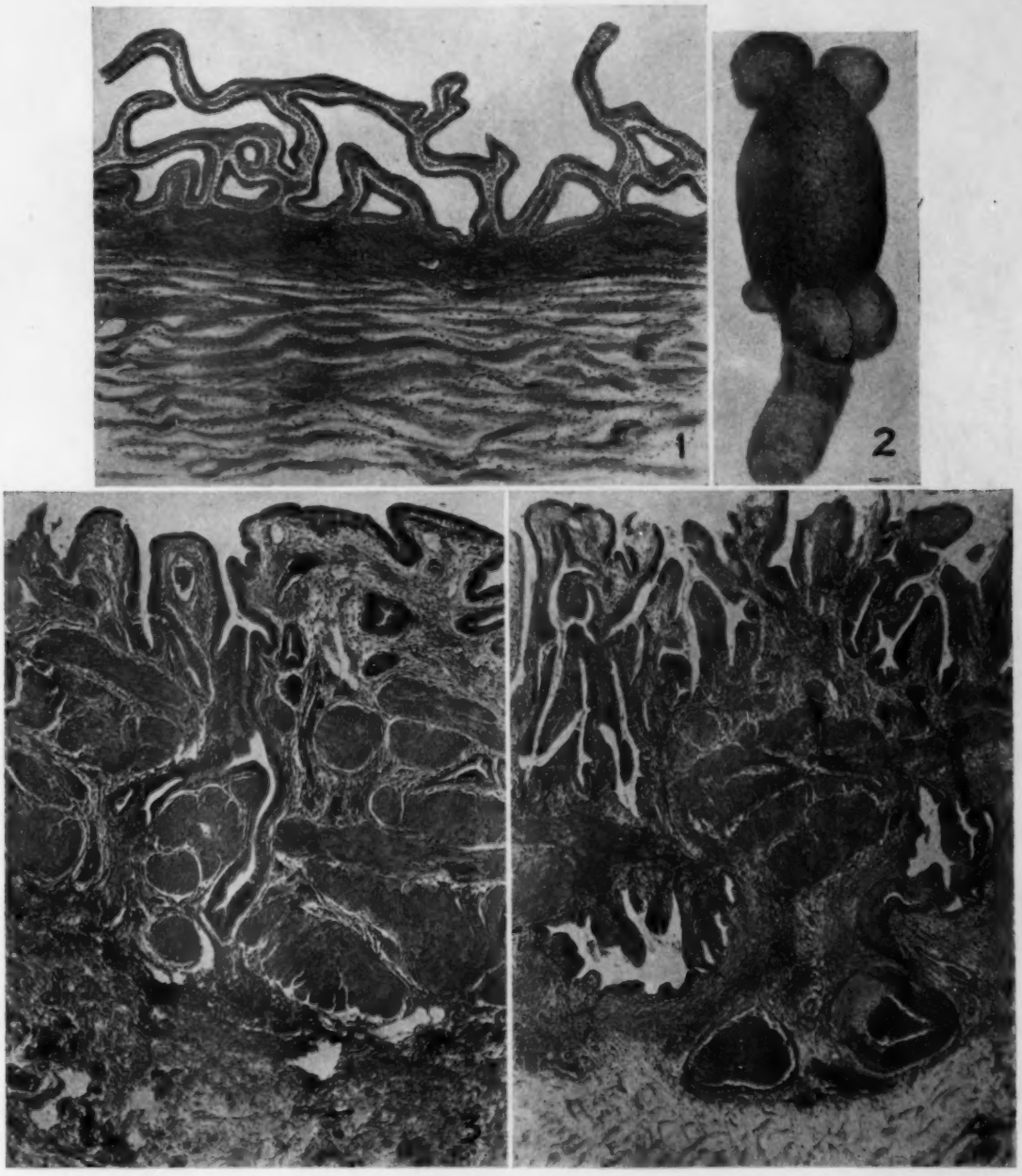


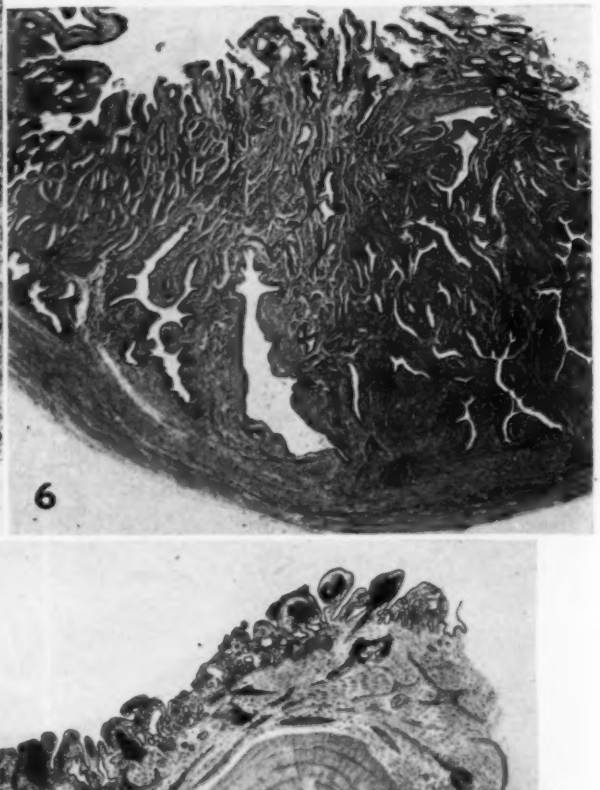
Fig. 1.—Characteristic villus formation (*Faltenbildung*) in the gallbladder of an adult. (From Aschoff and Bacmeister,[58] fig. 6.)

Fig. 2.—Gallbladder with intramural cysts, into which wax has been injected. (From Else.[69])

Fig. 3.—Chronic cholecystitis. Note Luschka crypts in the muscularis (× 35).

Fig. 4.—Chronic cholecystitis. A Luschka crypt extends through the muscularis (× 22).

sequence of crypt formation does not seem to be afforded any substantial support. That carcinoma can develop from the epithelial cells of the diverticula must be granted on a theoretic basis, so-called papillomas of the gallbladder and diverticula, although extensive papillary formations may occasionally themselves contain true crypt formation.



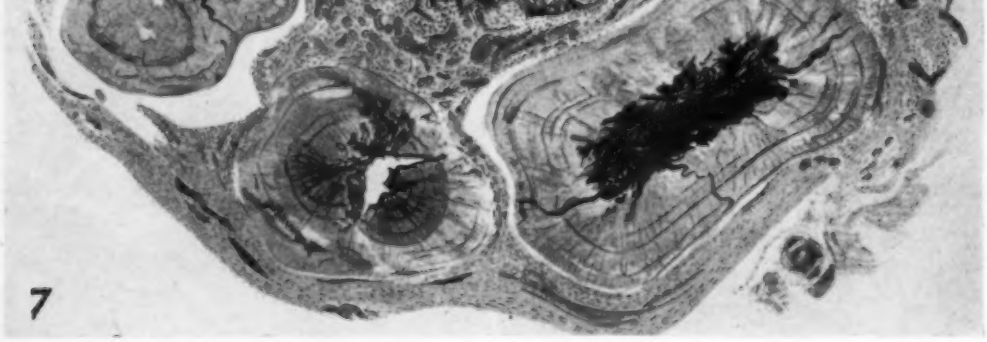


Fig. 5.—Chronic inflammatory cholelithiasis with new formation of Luschka's crypts in the inflamed gallbladder. (From Aschoff and Bacmeister,[58] fig. 32.)

Fig. 6.—Chronic cholecystitis. Note "adenoma" containing a multilocular Luschka crypt (× 13).

Fig. 7.—Intramural stones in former Luschka's crypts. (From Aschoff and Bacmeister,[58] fig. 38.)

but transitional stages are so infrequently observed that we are inclined to question any such significant role for these lining cells. Also we can see no sufficient reason to group together the

The supposition that gallstones in the lumen of the gallbladder contribute to the formation of diverticula is not proved. So-called normal gallbladders often contain well developed crypts. It

must be granted, however, that the neurogenic dysfunctional disturbances which may play a major role in the formation of diverticula might be linked with similar disturbances which produce gallstones. Likewise, the possibility that gallstones may occasionally be responsible for increased intracystic pressure must be admitted. But the main factors in the formation of crypts are advancing age plus the peculiarly loose and complex distribution of muscle bundles in the gallbladder wall, the isolated physiologic response of these bundles to normal stimuli and the increased pressure produced by dysfunctional or mechanical conditions.

Figures 1 to 7, inclusive, illustrate some of the gross and histologic features of diverticula of the gallbladder.

CONCLUSIONS

In approximately half of all gallbladders removed from persons more than 30 years of age the mucosa has invaginated the underlying structures, sometimes as far as the peritoneal lining.

This invagination tends to form diverticular spaces with branching pouches, which occasionally simulate mucous glands. They are lined with epithelium corresponding in every respect to that which lines the mucosa of the inner surface of the gallbladder. These cells secrete mucus or a mucus-like fluid.

The greater number of the diverticula open into the lumen of the gallbladder by ducts, which are often tortuous and narrow. Some are cut off in whole or in part from the lumen and become cysts with budlike branches. When such a group is more or less localized, a formation, often called an adenoma, is produced. It is most frequently found in the fundus of the gallbladder but may occasionally involve more, or even all, of the gallbladder. The term "adenoma" is misleading and inaptly applied. "Multicystic diverticula" and "multilocular cystic diverticula" are more fitting designations.

The crypts thus formed may contain bile, bile pigments, cholesterol crystals or at times typical biliary calculi. Exudative inflammation may occur in them up to the stage of abscess formation and even of rupture into the peritoneal cavity. The residuum of such inflammation may be obliteration of the epithelial lining, proliferation of connective tissue and collections of lymphocytes and other cells.

Increased intracystic pressure, absence of muscularis mucosae, a loosely, irregularly arranged muscular layer and the independent response of the muscle bundles to physiologic stimuli account for the initial diverticula-like indentations. The increased pressure is, more logically, the result of neurogenic dysfunctional states of the extrahepatic biliary system, although other mechanical factors, such as stones and inflammatory obstructions, may play etiologic roles.

Except for the complexity of the branches, these crypts correspond in every respect to the so-called false diverticula of the colon and the urinary bladder and should be denominated "diverticula of the gallbladder."

There is little except custom to justify calling them Luschka's crypts or Rokitansky-Aschoff sinuses. Not only did these investigators not possess sufficient priority but they failed in several important details to recognize the true significance of the structures which they described.

HISTOLOGIC CHANGES IN OVARY AND UTERUS OF LIVE DIROFILARIA IMMITIS RECOVERED FROM DOGS TREATED WITH TRIVALENT ANTIMONY COMPOUNDS

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The acquisition of filariasis by American troops has stimulated interest in the study of this disease in this country. Experimental study has been directed toward finding specific drugs that would be of value in the treatment of the disease as well as a method of treatment that would eliminate microfilarias from the peripheral circulation and thus be useful as a control measure.

Studies in the laboratory have made use of two naturally occurring filarial parasites in this country, Dirofilaria immitis in the dog and Litomosoides carinii in the cotton rat.

A number of authors have reported that various antimony compounds are effective in destroying the microfilarias in the peripheral blood of dogs infected with Dirofilaria immitis, but most have omitted microscopic studies of the adult parasites. Consequently, there is little evidence as to what effect the drugs have on the ovary and the uterine contents of the adult female Dirofilaria. Itagaki and Makino [1] in their study noted that there were no embryos in the uteri of worms recovered from the heart of one dog eight and a half months after the end of treatment with sodium antimonyl tartrate. Wright and Underwood,[2] using fuadin as the therapeutic agent, found no embryos in the uteri of adult worms recovered from dogs killed on or after the thirty-seventh day of treatment. Later, in a paper by Wright and Harwood,[3] the senior author reported that some of his observations seemed to indicate that the sterilization of the adult female worms was due to fatty degeneration and necrosis of the reproductive cells of the ovary. Brown and Sheldon [4] also were unable to find microfilarias in the uteri of adult females recovered alive from dogs treated with a combination of fuadin and sulfanilamide.

Recently Lawton, Brady, Ness and Haskins [5] reported on the elimination of the circulating microfilarias of Dirofilaria by 23 of 24 trivalent antimony compounds and the failure of three mercury compounds to effect such a result. In the course of our studies, adult female dirofilariae were available for histologic examination. The present report is based on the examination of 25 of these worms recovered from dogs treated with various antimony compounds and of 2 from dogs treated with mercury compounds.

METHODS

Microfilaria counts of blood samples of the treated dogs were made each time the drug was administered (six days a week). In those instances in which the count never reached zero, the drug was administered until the day of autopsy. In those in which treatment effected a disappearance of microfilarias from the blood, the administration of the drug was discontinued when microfilarias were no longer found. The blood was

From the Pathology and Zoology Laboratories, National Institute of Health.

1. Itagaki, S., and Makino, R.: J. Jap. Soc. Vet. Sc. **6**:1, 1927.

2. Wright, W. H., and Underwood, P. C.: Vet. Med. **29**: 234, 1934.

3. Wright, W. H., and Harwood, P. D., in Christie, J. R.: An Introduction to Nematology, Babylon, N. Y., M. B. Chitwood, 1941. sect. 2, pt. 2, p. 322.

4. Brown, H. W., and Sheldon, A. J.: J. Am. Vet. M. A. **98**:477, 1941.

5. Lawton, A. H.; Brady, F. J.; Ness, A. T., and Haskins, W. T.: Tests of Mercury and Antimony Compounds in Dirofilaria Immitis and Litomosoides Carinii Infections, Am. J. Trop. Med. **25**:263, 1945.

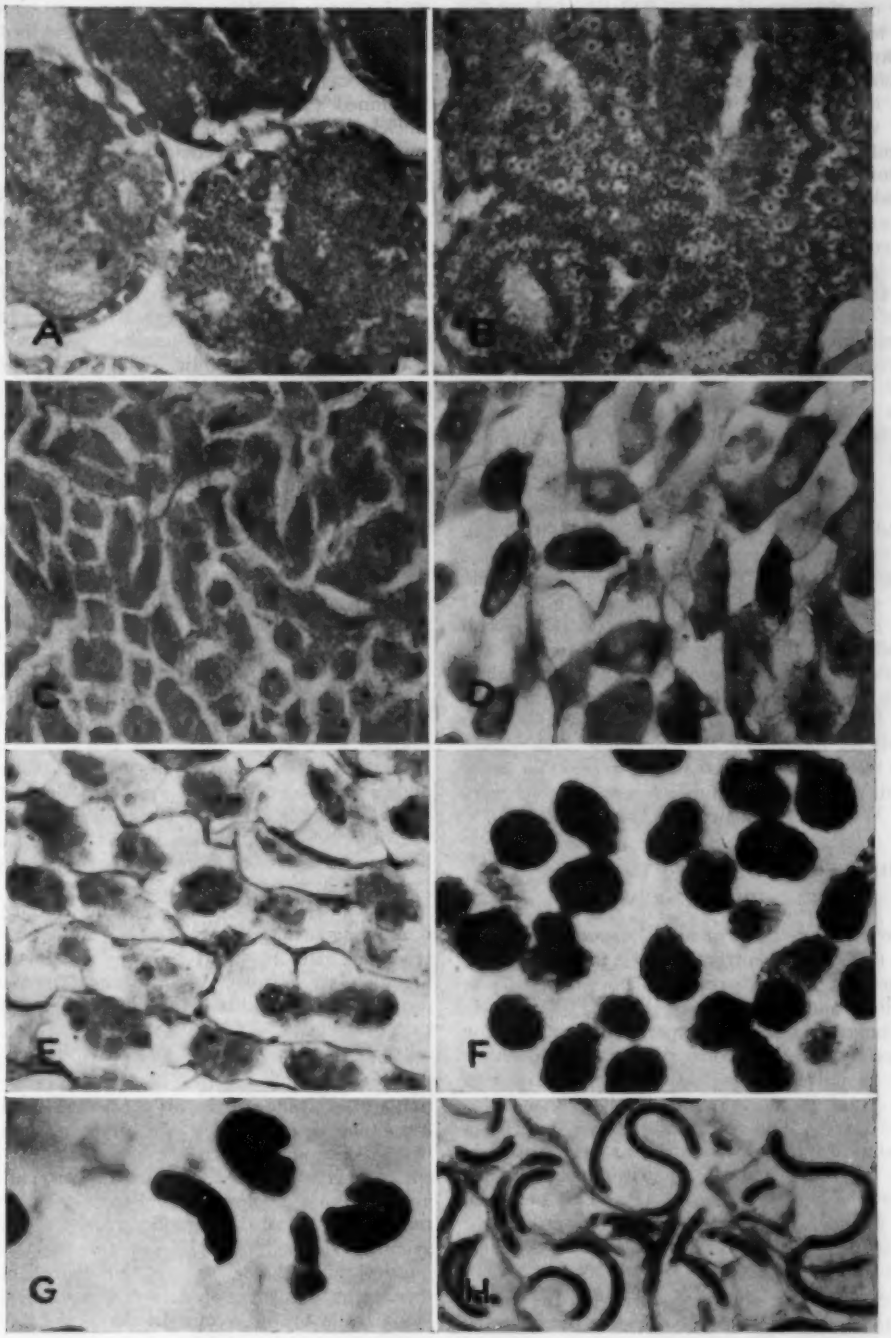

Fig. 1.—Normal Dirofilaria immitis. The sections from which all these photomicrographs except *C* were made were stained with hematoxylin azure eosinate. The section from which photomicrograph *C* was made was stained with hematoxylin.

A, cross section of the posterior portion of a female, showing multiple ovarian convolutions. × 250.

B, ovary showing rosette arrangement of the ova. Note the pale nuclei and the distinct nucleoli. × 500.

C, section of an ovary with the ova more mature. × 500.

D, unicellular and biocellular ova in the first (posterior) part of the uterus. One ovum shows beginning mitotic division. × 500.

E, ova in relatively early stages of cell division. Note mitoses. × 500.

F, ova formed of numerous cells having deeply basophilic nuclei and cytoplasm, giving an almost opaque mass. × 500.

G, developmental stage (fissure formation) showing early differentiation of microfilarias. × 500.

H, well developed microfilarias. × 500.

thereafter examined at weekly intervals until the termination of the experiment. This period varied from three to one hundred and seven days (average, sixty-two days).

Fifteen female worms served as controls for this study. Ten of these were recovered from 5 untreated dogs and 5 worms were recovered from 2 dogs that died two and a half hours after administration of a single dose of a drug under test.

After removal of the worms from the heart or the pulmonary arterial system at autopsy, one random female was coiled around a glass tube, the ends of the worm were secured by small rubber bands, and the preparation fixed in solution of formaldehyde U. S. P. (1:10). Before immersion in the fixative, the body wall was punctured at a few points in an attempt to facilitate penetration of the fixative. However, in the later part of the study it became evident that unpunctured worms appeared as well fixed as those punctured, and the practice was discontinued. This eliminated the disadvantage of a loop of the genital tract herniating through the puncture in the body wall. The filarias were dehydrated in acetone, cleared in benzene and cedar oil and infiltrated with and embedded in 56 C. paraffin. The rubber bands were removed and the coiled filaria was slipped from the glass tubing just previous to embedding. The worm was oriented in such a way that sections through the axis of the coil could be made. By this method cross sections of the worm at 11 mm. intervals were obtained. In sectioning, in some instances, the body wall and the viscera of the worm were fractured or distorted. This appeared to be due largely to incomplete paraffin infiltration. Sections of each worm were stained with hematoxylin azure eosinate [6] and Van Gieson's stain. A few sections were stained with Weigert's iron hematoxylin.

MICROSCOPIC EXAMINATION OF ADULT DIROFILARIA

In order to compare the ova and the microfilarias in worms from treated and untreated dogs, the appearance of these structures in the normal female worm will be briefly described and illustrated.

In the technic used in this experiment, an anterior ovary was not seen in any worm recovered from either a treated or an untreated dog. Consequently any reference to an ovary in the following description denotes the posterior ovary. The term "uterus" or "uteri" as used here refers to these structures as seen in cross section, with no attempt made to distinguish between an anterior and a posterior organ. As used here the word "ova" includes both the unicellular reproductive cells, chiefly in the ovary, and the multicellular developmental forms found in the uterus.

Normal Worm.—In the posterior sections of the worm, from three to seven convolutions of the ovary were seen in cross section (fig. 1 *A*). Thus, in one level of the worm, unicellular ova of varying degrees of maturity were seen. The ova were first arranged in solid cellular cords. In higher levels the cells were grouped in multiple rosettes (fig. 1 *B*). In the anterior portion of the ovary the cells again appeared diffusely or irregularly disposed. With this progression forward, cells became altered in size and shape: small and polygonal at first, large and oval or spindle-shaped in the higher levels. The mature unicellular ova varied in size up to 17 microns in their widest diameter, and had nuclei measuring about 6 microns. The nuclei were oxyphilic and had deeply basophilic large nucleoli (up to 2 microns) (fig. 1 *C*). In the first portion of the uterus unicellular ova were seen which were not greatly different from those just described (fig. 1 *D*); however, ova showing 5 to 7 cells (in one optical plane) were occasionally present in the next level of the worm examined (11 mm. distant). With this cell cleavage, nuclei were reduced in size (3 to 4 microns) and had a moderate amount of reticulated basichromatin (fig. 1 *E*). Nucleoli measured only 0.5 to 0.7 micron. The formation of new cells, which takes place by mitotic division, did not add greatly to the bulk of the developing ova (compare fig. 1 *D* to *F*). In ova showing about 20 cells in one optical plane, the nuclei were about 2 microns in diameter and had much deeply basophilic chromatin; the nucleoli were no longer visible. In this stage the cytoplasm, although very small in amount, was also deeply basophilic. This combination of nuclear and cytoplasmic basophilia prevented satisfactory study of the cell components (fig. 1 *F*). Hematoxylin stain was used in determining nuclear size in this developmental stage. The fissure which develops in these masses and the well formed microfilarias which differentiate from these structures are illustrated in *G* and *H* in figure 1. The microfilarias in various stages of development were usually seen in the genital tract in the anterior one third and one half of the worm. The level in which they were found, however, was somewhat variable as was the number of microfilarias present; in some the uteri were filled.

It is evident from a study of some of the worms from untreated dogs that abnormalities due to senility or other causes occur. Thus in 1 dog 3 female dirofilarias showed on gross inspection a yellowish discoloration in contrast to the normal white translucent appearance. Two of these were microscopically abnormal. One showed an empty uterus. The uterus of the other worm contained from few to moderate numbers of abnormally developed or degenerating forms in addition to many normal multicellular ova. Two grossly normal worms from another dog showed no developing ova in most levels, although microfilarias were present in anterior levels of the genital tube. One grossly normal worm from a third dog showed no microfilarias, although multicellular ova were seen in the uterus.

Worms from Treated Dogs.—In the most severely damaged worms, all levels of the uterus were empty or at most contained in an occasional level one to few unicellular ova or ova in which only a few cell divisions had taken place. These cells showed degenerative changes, characterized by markedly oxyphilic cytoplasm and pyknotic nuclei. Some were necrotic and were represented by oval oxyphilic masses without nuclei or granular debris (fig. 2 *C* and *D*). In some of these multicellular masses, seen in the more anterior levels, there was evidence of retarded development of individual cells of the ovum. In these one or more of the nuclei were relatively large and pale, a condition normal to cells in the posterior portion of the uterus. It should be pointed out here that uninuclear ova or ova composed of only a few cells were seen rarely in the intermediate and anterior levels of the uterus in grossly normal worms from untreated dogs. Also, in these worms, occasional degenerating forms were seen, but in comparison with the large number of normal ova they were rare.

The condition of the ovaries in the severely damaged worms was variable. In a moderate number, from few to many of the ova showed oxyphilic hyaline cytoplasm or were frankly necrotic. These changes occurred more frequently in the mature ova than in those in the posterior part of the ovary (fig. 2 *A* and *B*). Either

6. Lillie, R. D.: Stain Technol. **16**:1, 1941.

with or without observed changes in the ovary, the first portion of the uterus often contained degenerating or necrotic uninuclear and binuclear ova, which were sometimes recognizable only by shape and size and their location in the uterus.

As mentioned earlier, a characteristic stage in the development of the ovum is one in which the entire multicellular mass is intensely basophilic when stained with azure eosinate buffered at p_H 4.5, our routine p_H for vertebrate tissue fixed in neutral solution of formaldehyde U. S. P. (1:10). This stage was not seen in the worms just described. However, somewhat similar stages were seen in 2 worms from 2 treated dogs. In these worms a very few multicellular ova were present. They varied from normal in that the cell cytoplasm was moderately eosinophilic and the nuclei were small and pyknotic. Occasionally one or more of the constituent cells showed karyorrhectic changes (fig. 2 *E*). It appears certain that these forms were derived from those described as being intensely basophilic and dif-

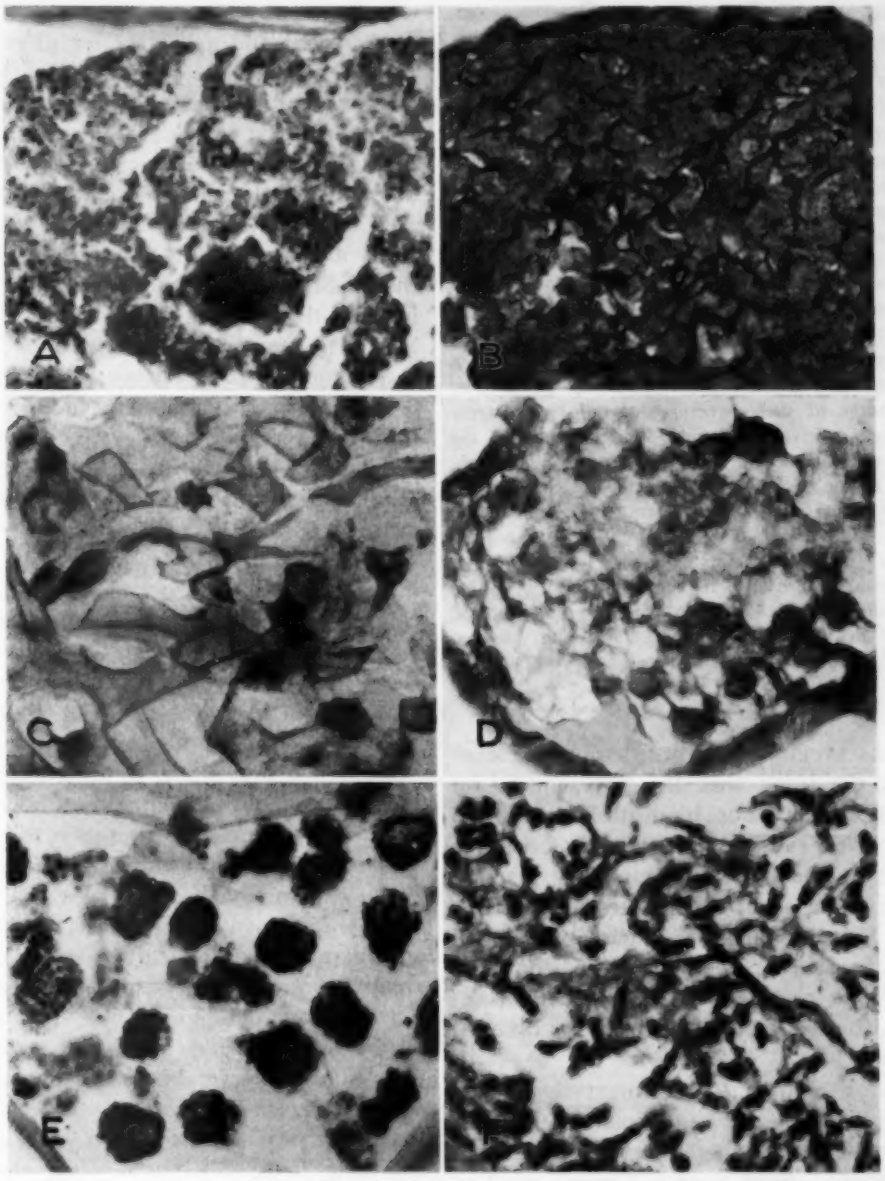

Fig. 2.—Dirofilaria immitis from treated dogs. All sections were stained with hematoxylin azure eosinate.
A, ovary showing karyorrhectic necrosis. Compare with figure 1 *B*. × 500.
B, ovary showing coagulation necrosis. Compare with figure 1 *C*. × 500.
C, abnormal unicellular ova in the uterus. Compare with figure 1 *D*. × 500.
D, degenerating ova which have undergone only a few cell divisions. Compare with figure 1 *E*. × 500.
E, ova formed of numerous cells. Note the abnormal distribution of nuclei. The cytoplasm of these cells is oxyphilic. A few abnormal ova formed of only a few cells are seen at the left. Compare with figure 1 *F*. × 500.
F, degenerating microfilarias. Most uteri of worms from treated dogs did not contain microfilarias. Compare with figure 1 *H*. × 500.

fered from them only in minor degree. That this alteration represents early degeneration seems certain, but it could not be determined from this material whether or not these changes are reversible.

The abnormal findings described in the foregoing paragraphs were based on the examination of 18 adult female Dirofilaria immitis parasites. Microfilarias were not found in any of these worms, and the microfilaria count of the peripheral blood of 16 of these dogs had, under treatment, dropped to zero from fourteen to one hundred and seven days (average, sixty-eight days) before autopsy. The peripheral blood was not examined in 1 dog, and the microfilaria count of another dog was 14 per 20 c. mm. at death.

Of the 27 worms examined in this study, 9 showed from few to many microfilarias in the anterior portions of the genital tube. The microfilarias in 6 of these showed degenerative changes ranging from slightly increased cytoplasmic oxyphilia and nuclear pyknosis to actual necrosis and fragmentation; the latter was seen in only 1 worm (fig. 2 *F*). Normally developed, intensely basophilic multicellular ova were absent from 7 of the 9 worms, but their altered counterparts (already described) were present in moderate numbers in various uterine levels. In addition to these changes, 3 worms showed necrosis of unicellular ova and of ova which had undergone only a few cell divisions (fig. 2 *D*). The ovaries of some of these worms showed slight variation in cytoplasmic staining and indistinctness of the nuclei and nucleoli of the ova. These changes were considered minor and of undetermined significance. Two worms from 2 dogs of this group were normal in all respects. These dogs were treated with compounds of mercury.

Microfilarias were found in the peripheral blood of 6 of the 9 dogs of this group at the last examination made before death of the animal. The counts varied from 27 to 2,112 per 20 c. mm. of blood (average, 60).

COMMENT

There has accumulated considerable evidence to support the belief that the late sequelae of human filariasis are due to death of the adult parasites. This evidence suggests that there is an increase in the host's inflammatory reaction due to the liberation of toxic materials from the parasites. If such be the case, it has been pointed out that a therapeutic agent that killed the adult parasites might cause severe anaphylactic reactions and also accelerate the onset of the dreaded complications.

However, a method of treatment that could render the human host noninfectious for the mosquito intermediate host might serve as a valuable control measure. Such a method would need to eliminate the circulating microfilarias and also prevent the production of microfilarias by the live adults.

The present study shows that at least temporary sterilization of the females of Dirofilaria immitis in dogs can be accomplished with a number of antimony preparations. Eight of these sterilized worms were recovered from 8 dogs that had not received treatment for seventy days or more; 2 of these had received no treatment for one hundred and four and one hundred and seven days. Longer periods of observation, however, would be necessary to determine whether or not the sterilization is permanent. Brown [7] recently reported on persons infected with Wuchereria bancrofti who were treated with lithium antimonyl thiomalate. The microfilaria count of these patients was reduced 85 to 100 per cent in four and a half to five months after the last treatment. He considered it likely that the adult worms had been killed, since microfilarias were not being produced four and a half to five months after the last treatment. However, it is possible that the adult worms were sterilized but not killed. Assuming that the results of our study are applicable to filarial infections of man, the finding of sterility in live adult Dirofilaria immitis three and a half months after the last treatment supports such a thesis.

The most striking changes in the genital tracts of these worms were absence of microfilarias and necrosis or earlier degenerative changes of unicellular ova and ova in relatively early stages of segmentation. Degenerative changes and actual necrosis of portions of the ovary, however, were seen in a number of worms. The absence of microfilarias and of normally developed multicellular ova might be explained as a result of the direct effect of the drug on these stages. It seems more likely from the present experiment that in most instances the absence of the microfilarias is brought about by injury at earlier stages, thus preventing further normal development. It should be pointed out, however, that a few worms showed degeneration of microfilarias within the genital tube of the adult worm.

An explanation is needed for the presence of degenerating ova in the uterus of the worm for considerable periods after discontinuance of treatment (up to one hundred and seven days). That necrotic debris is so long in being removed seems unlikely, and also some cells, although necrotic, had maintained their form, indicating that the degeneration was recent. It is possible that the ovary was injured to such a degree by the drug that, although ova are shed into the uterus, they are incapable of normal development and differentiation, and ultimately degenerate. A third possibility to be considered is that the chemical is retained for a considerable period by the filarias and that the ovary and the uterine contents continue to be influenced by the drug for some time after treatment is discontinued.

7. Brown, H. W.: J. A. M. A. **125**:952, 1944.

Which, if any, of the suggested explanations is correct cannot be determined from the present material, but further study is planned, which should furnish additional data on this point.

There was complete agreement between the presence or the absence of microfilarias in the blood and the presence or the absence of microfilarias in the uteri of worms from 22 treated dogs. There was no agreement in 4 dogs. One of these dogs had a microfilaria count of 14 at death and the single female worm found at autopsy showed an empty uterus in all levels. There were 3 dogs in which the microfilaria count had been 0 for three, eighteen and seventy-three days, but microfilarias were present in the uteri of the recovered worms. This finding leads to the suggestion that histologic examination of the female worms should be an essential part of any experimental study in which an effective therapeutic agent is being sought.

SUMMARY

Live adult females of Dirofilaria immitis recovered from 25 dogs treated with various trivalent antimony compounds were examined histologically mainly to determine the effect of the drug on the ovary and the uterine contents. Therapeutically active compounds produced striking changes in the ovary and the uterine contents which in most cases agreed well with the microfilaria counts of the circulating blood. The changes were early degeneration or necrosis of ova and, in most worms, absence of microfilarias. The uteri of some worms were entirely empty, and areas of necrosis were evident in the ovaries. Worms from 2 dogs treated with mercury compounds were normal. In view of these observations it is suggested that histologic examination of female worms recovered alive should be a part of any experimental study to determine the effectiveness of a chemotherapeutic agent.

RENAL INSUFFICIENCY ASSOCIATED WITH LYMPHOPENIA AND ATROPHY OF LYMPHATIC TISSUE IN MAN

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INTRODUCTION

In a recent publication[1] evidence was presented that acute renal insufficiency in adult dogs is regularly associated with lymphopenia and atrophy of lymphatic tissue. This evidence consisted of (1) a definite reduction in the gross weight of the spleen and the popliteal lymph nodes, (2) a sharp decrease in the total number of circulating lymphocytes, to 32 per cent of the control level, and (3) gross and histologic changes in the spleen, the thymus, Peyer's patches, solitary follicles and lymph nodes, which were interpreted as atrophy due to exhaustion. The principal histologic changes in the lymph nodes were a general "washing out" of lymphocytes from the cortex and a proliferation of reticular cells in what were previously germinal centers — a change referred to as "reticular arrest." These atrophic changes in the lymphatic tissues were independent of diet and of the manner in which renal insufficiency was produced (by poisoning with uranium nitrate or mercury bichloride or by bilateral nephrectomy). The changes were observed as early as four days after renal injury, and dogs that survived the renal injury due to heavy metal gave evidence that the changes were reversible. The changes seemed to be related directly to renal insufficiency and not to indirect effects such as inanition, acidosis and infection. The suggestion was offered that the kidney normally elaborates (or concentrates) a "lymphocytogenic" substance and that following renal insufficiency the source of this substance is no longer available.

CASES STUDIED

These experiments suggested the question: Are lymphopenia and atrophy of lymphatic tissue present in any human cases of renal insufficiency? A survey of the problem indicated that pertinent data could be obtained from three sources: (1) hospital records filed under selected headings (see table 1 and the brief protocols of 4 cases in the appendix), (2) cases with adequate records of renal insufficiency in which necropsy had been performed (table 2), and (3) current cases of renal insufficiency (table 3).

Analysis of the clinical and necropsy data collected in human cases of renal insufficiency indicates that in a limited number of cases lymphopenia and atrophy of lymphatic tissue have occurred. Massive damage to the renal cortex in a relatively short period is the factor common to most of the human patients — and to the experimental animals.

TABLE 1.—*Review of Hospital Records for Human Cases of Renal Insufficiency Associated with Lymphopenia and Atrophy of Lymphatic Tissue*

Diagnosis Under Which Cases Were Filed	Cases	Number with Lymphopenia or Atrophy of Lymphatic Tissue, or Both
Mercury bichloride poisoning	20	2*†
Glomerulonephritis and nephrosclerosis	15	0
Lipoid nephrosis	1	1†
Third degree burns	10	1†
Diabetic acidosis and coma	10	0
Bilateral pyelonephritis	6	2
Total	62	6

* These were the only cases in this group that proved fatal.
† The clinical history is abstracted in the appendix.

COMMENT

The data presented in the tables and the appendix are probably representative of those that can be obtained in a medium-sized hospital (225 beds), but they are too meager and incomplete for any detailed analysis or extensive discussion. They suffice to show that human cases of renal insufficiency associated with lymphopenia are not difficult to find.

A few general comments are suggested by these studies: 1. Evidence of atrophy of lymphatic tissue in man is not as convincing as that obtained in dogs.[1] There are at least two reasons for this: (*a*) Quantitatively, lymph nodes in any given region in man are more apt to be variable

This investigation was aided by a grant from the John and Mary R. Markle Foundation.

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1. Holman, R. L.: Arch. Path. **37**:124, 1944.

and multiple, and the weight of true lymphatic tissue in any given lymphoid organ, especially in the spleen, is more likely to be obscured by congestion and reticuloendothelial hyperplasia. (*b*) Qualitatively, the lymphatic tissues in man do not display with any degree of regularity the changes described in dogs as "reticular arrest." Despite these handicaps, the changes in the lymphatic tissues in man are in the same general direction as those in the dog, namely, atrophy; in a few cases histologic changes similar to those described and illustrated in dogs [1] were observed in man (figs. 1 to 3).

2. Emphasis in human cases of renal insufficiency should be placed on lymphopenia.

3. In all the cases with lymphopenia (less than 1,000 lymphocytes per cubic millimeter)—6 in table 1, 5 in table 2 and 5 in table 3—there probably was extensive damage of the proximal convoluted tubules occurring in a relatively short period (three to eleven days).

4. Renal disease with predominantly glomerular damage (glomerulonephritis) and that with predominantly vascular damage (nephrosclerosis) appear not to be associated with lymphopenia.

5. Slowly developing renal insufficiency such as that in patients with urinary obstruction without infection (e. g., hydronephrotic atrophy) is not accompanied by lymphopenia.

TABLE 2.—*Summary of All Cases with Adequate Records of "Azotemia" * in Which Necropsy Had Been Done During a Five Year Period* †

Sex	Age	Principal Diagnosis	Date of Admission	Maximum Blood Urea Nitrogen (Mg. per 100 Cc.) and Date	Last Recorded Lymphocyte Count (Total Number per Cu. Mm.) and Date	Date of Death	Weight of Spleen, Gm.	Lymph Nodes Examined ‡
				Group 1. Chronic Glomerulonephritis				
M	32	Chronic glomerulonephritis	11/15	125 (12/1)	1,287 (11/26)	12/ 4	180	Peripancreatic, mesenteric
				Group 2. "Nephrosis"				
F	28	Staphylococcic septicemia, "sulfathiazole nephrosis"	11/ 6	84 (11/18)	1,035 (11/17)	11/18	...	Peripancreatic, perigastric
F	29	Staphylococcic septicemia, "sulfathiazole nephrosis"	7/20	78 (8/19)	3,652 (8/19)	8/19	150	Periaortic, peripancreatic, pelvic
				Group 3. Nephrosclerosis				
M	42	Malignant hypertension	1/26	75 (1/27)	1,428 (1/26)	2/2	...	Peripancreatic, perigastric
M	50	Hypertensive cardiovascular renal disease (afebrile)	7/20	150 (8/2)	1,461 (7/20)	8/3	195	Periaortic, peripancreatic
M	58	Hypertensive cardiovascular renal disease, pneumonia	8/12	128 (8/12)	1,554 (8/12)	8/13	90	Periaortic, peripancreatic, inferior cervical
M	34	Malignant hypertension	11/27	240 (11/30)	.....	12/4	290	Peripancreatic, trachebronchial
				Group 4. Acute Bilateral Pyelonephritis				
F	51	Diabetes, pelvic abscess, bilateral pyelonephritis	2/20	90 (3/21)	**292** (3/21)	3/21	...	Peripancreatic
M	36	Giant renal calculi, bilateral pyelonephritis	4/20	92 (5/6)	**910** (4/22)	6/20	250	Peripancreatic, periaortic, inferior cervical
F	73	Carcinoma of left ureter, bilateral pyelonephritis	6/20	68 (7/3)	**605** (7/5)	7/14	285	Peripancreatic, inferior cervical
M	3	Congenital urethral valves, bilateral pyonephrosis	4/17	60 (4/20)	3,000 (4/17)	4/21	...	Periaortic, pelvic
M	65	Urethral stricture, bilateral pyelonephritis	7/20	87 (7/20)	.....	7/21	300	Periaortic, peripancreatic
				Group 5. Infections (Systemic)				
M	72	Staphylococcic abscesses of lungs and kidneys	11/25	80 (11/27)	1,740 (11/25)	12/1	360	Peripancreatic
M	65	Perforated gastric ulcer, generalized peritonitis	10/16	86 (10/19)	**654** (10/19)	10/20	50	Periaortic, peripancreatic
M	36	Subacute bacterial endocarditis, embolic glomerulonephritis	12/5	280 (12/26)	1,970 (12/31)	1/1	360	Periaortic, peripancreatic
M	64	Perforated duodenal ulcer, subhepatic abscess	2/11	80 (3/3)	1,100 (2/28)	3/4	150	Periaortic, mesenteric
M	16	Stab wound of abdomen, generalized peritonitis	3/3	93 (3/18)	**343** (3/12)	3/18	180	Periaortic, inferior cervical, mesenteric
				Group 6. Miscellaneous				
M	48	Congenital polycystic kidneys	7/11	122 (8/8)	1,120 (7/11)	8/9	130	Periaortic, peripancreatic, tracheobronchial
F	78	Mild chronic nephritis, acute necrotizing aortitis	7/8	60 (7/8)	2,912 (7/8)	7/8	110	Periaortic, perigastric, inferior cervical

* Azotemia is arbitrarily defined as a condition characterized by more than 60 mg. of urea nitrogen per hundred cubic centimeters of blood or more than 100 mg. of nonprotein nitrogen per hundred cubic centimeters of blood.

† The 19 cases summarized constitute 6.7 per cent of the 284 cases in which necropsy was performed (exclusive of cases of prematurity and stillbirth) during the period from 1939 to 1944. All the patients were white.

‡ Most of these 42 lymph nodes were obtained from material fixed in Kaiserling solution. Microscopic sections were made of each node.

6. None of the cases of strictly unilateral damage of the kidney exhibits lymphopenia.

7. With recovery from renal damage the lymphopenia is reversible (cases 1 and 3, table 3).

The possible significance of these observations is not as yet apparent. Further studies may show that the curve of the total number of circulating lymphocytes per cubic millimeter may prove to be a valuable adjunct to the degree of azotemia in prognosticating the outcome of certain cases of renal insufficiency. It is further conceivable that blood lymphocyte counts may prove worth while in establishing the diagnosis of nephrosis and differentiating this from other diseases of the kidney.

The evidence that the lymphopenia associated with renal insufficiency in some patients is due to a deficiency of something normally derived from the kidney rather than to an excess of a "toxic factor" derived from the damaged kidney rests on the results obtained following bilateral nephrectomy in dogs.[1] The possibility that the lymphopenia results from failure of elimination of a normal metabolite is not excluded by these studies, but this seems improbable, especially since the atrophic changes in the lymphatic system are not related to diet, infection, acidosis or other known metabolic change.

The next logical step in these studies would be to try to isolate from the renal cortex and concentrate an active principle that stimulates the production of lymphocytes. I have been reluctant to deviate from the main problem (the relationship of diet and renal insufficiency to arterial disease) which yielded the observations in this and the previous [1] papers, for I am aware of all the control data that would have to be obtained to establish a "hormone." It is hoped that some one will make the attempt.

TABLE 3.—*Summary of Current Cases of Azotemia* *

Case	Sex	Age	Clinical Diagnosis	Blood Urea Nitrogen (Mg. per 100 Cc.) and Date	White Blood Cell Count (Total Number per Cu. Mm.) and Date	Lymphocytes, per Cent	Total No. of Lymphocytes, per Cu. Mm.	Comment
1	M	10	Acute nephritis 10 days after scarlet fever	80 (2/10) 28 (3/8)	8,000 (2/10) 6,750 (2/15) 9,700 (7/25)	12 10 45	960 675 4,365	Blood pressure 160 systolic and 110 diastolic; patient discharged improved; readmitted for operation for hernia, blood pressure 126 systolic and 86 diastolic
2	M	80	Prostatic hypertrophy, bilateral pyelonephritis, uremia	140 (6/17)	16,600 (6/17)	1	166	Patient died; necropsy refused
3	F	36	Bilateral pyelonephritis with anuria	74 (8/30) 85 (9/3) 56 (9/5) 13 (9/19)	22,900 (8/27) 12,300 (8/29) 13,000 (8/30) 14,200 (9/8) 13,700 (9/18)	5 9 4 13 25	1,145 1,107 520 1,846 3,425	Staphylococcus and diplostreptococcus cultured from each ureteral specimen; patient discharged improved 9/21
4	F	16	Acute nephritis superimposed on chronic nephritis	24 (8/2) 40 (8/9) 62 (8/23)	4,350 (8/1) 12,800 (8/9) 16,000 (8/13) 11,550 (8/23)	27 6 15 25	1,175 768 2,400 3,880	Phenolsulfonphthalein test 12 per cent in 2 hours; blood pressure 180 systolic and 140 diastolic; marked edema; death 9/7; necropsy refused
5	M	40	Urinary calculi following automobile accident 5 years previously; chronic bilateral pyelonephritis	144 (8/9)	6,600 (8/3) 8,800 (8/9)	20 16	1,320 1,408	Death 8/13; necropsy refused
6	F	44	Cardiovascular-renal disease, uremia	135 (9/30) 100 (10/2)	16,600 (9/30)	6	996	Blood pressure 210 systolic and 100 diastolic; hemoglobin content 6.4 Gm.; red cell count 2,250,000; death 10/3; necropsy refused
7	M	22	Acute poisoning due to unknown agent, anuria	32 (1/9) 54 (1/11) 88 (1/13) 120 (1/15)	29,300 (1/9) 22,600 (1/13) 21,000 (1/15)	5 5 2	1,465 1,130 420	Death 1/16; necropsy obtained. See case 2 in appendix
8	M	24	Congenital hypoplasia of kidneys, chronic uremia	128 (3/1) 175 (3/4) 105 (3/13)	5,500 (3/1) 11,700 (3/4) 4,500 (3/14)	27 14 41	1,485 1,638 1,845	Phenolsulfonphthalein test 0 per cent in 2 hours; blood pressure 170 systolic and 110 diastolic; patient discharged unimproved 3/16
				320 (10/4)	8,900 (10/4)	14	1,246	Patient discharged unimproved 10/11

* Azotemia arbitrarily defined as a condition characterized by more than 60 mg. of urea nitrogen per hundred cubic centimeters of blood or more than 100 mg. of nonprotein nitrogen per hundred cubic centimeters of blood.

SUMMARY

Data were published last year [1] which indicate that acute renal insufficiency in dogs is regularly associated with lymphopenia and atrophy of lymphatic tissue. To determine whether or not a similar association occurs in man, three types of source material were analyzed: (1) hospital records filed under selected headings, (2) all cases with adequate records of azotemia in which necropsy was done during a five year period and (3) current cases of azotemia during a period of one year.

Isolated instances of lymphopenia or atrophy of lymphatic tissue or both were found in each of the three groups of cases studied. The total of 16 such instances represents 19 per cent of the total number of cases studied.

There is nothing in the data from the human cases presented in this paper that is incompatible with the original suggestion that a "lymphocytogenic substance" is normally elaborated by the renal cortex. When the source of this hypothetic substance is destroyed, atrophy of lymphatic tissue and lymphopenia ensue.

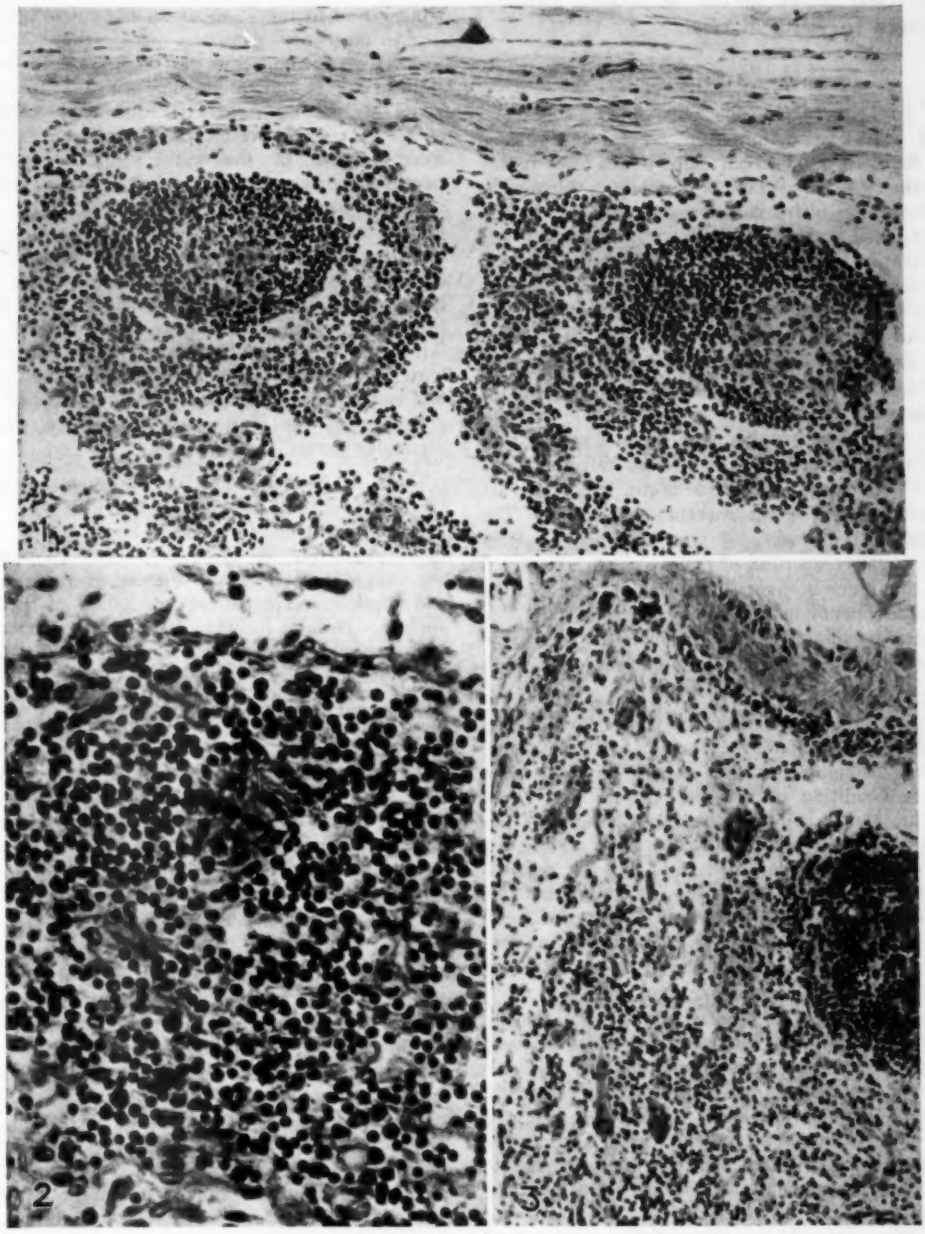

Fig. 1 (case 2, table 2, group 4).—Inferior deep cervical lymph node. Note the depletion of cortical lymphatic tissue. Two of the former follicles show "reticular arrest." Hematoxylin and eosin; × 164.

Fig. 2 (case 2 in the appendix).—Mesenteric lymph node showing a small area of "reticular cells" in what was previously a germinal center. Hematoxylin and eosin; × 350.

Fig. 3 (case 3 in the appendix).—Inferior deep cervical lymph node showing early "washing out" of lymphocytes from the cortex and an inactive germinal center. Hematoxylin and eosin; × 164.

The factor common to all of these isolated human cases and to the experimental cases is extensive damage of the renal cortex (proximal convoluted tubules) in a relatively short period.

Human subjects are not as satisfactory as dogs for evaluating atrophy of lymphatic tissue on

anatomic grounds; hence in regard to man the chief reliance must be placed on lymphopenia.

Two potential applications of these studies, namely, prognosticating the outcome of cases of mercury bichloride poisoning and differentiating nephrosis from other forms of kidney disease, are suggested for clinical trial.

APPENDIX

CASE 1 (mercury bichloride poisoning).—A white man of 59 years was admitted to Watts Hospital at 4 p. m. of Sept. 15, 1943, about eight hours after taking ten 7½ grain (0.48 Gm.) tablets of mercury bichloride. He was vomiting blood and passing grossly bloody urine. The usual treatment of gastric gavage followed by administration of milk, sodium bicarbonate and sodium formaldehyde sulfoxylate was given before he was admitted to the ward. The temperature was 102 F.; the pulse rate, 86; the respiratory rate, 20. Examination of the urine revealed a heavy cloud of protein and many red blood cells. Routine studies of the blood the following morning showed a total white cell count of 14,100 with 4 per cent lymphocytes (564 per cubic millimeter). No studies of blood nonprotein nitrogen were made. The patient died thirty-six hours later, fifty-six hours after ingestion of the mercury bichloride. The anatomic diagnosis at necropsy was: corrosive gastroenteritis, coagulative necrosis of the proximal convoluted tubules of the kidneys, acute hemorrhagic pancreatitis and hypostatic pneumonia (due to aspiration?). The necropsy data in this case are incomplete. No gross tissue was available for study and the microscopic studies did not include any lymphatic tissues.

CASE 2 (poisoning due to mercury ?).—A white man 22 years of age, a meat cutter, known to be a "heavy drinker," was brought into the emergency room in a semicomatose condition by friends. About two hours previously he had taken an unknown amount of an unidentified poison referred to as Blue Star. The temperature was 97.6 F.; the pulse rate, 95; the respiratory rate, 26. The blood pressure was not obtainable. He was vomiting a pinkish red mucoid material with a strong odor of alcohol (wine ?). There was moderate generalized abdominal tenderness but no other findings of note.

No urine was passed in the next seven days before he died. His blood urea nitrogen rose progressively from 32 mg. on the day of admission to 120 mg. per hundred cubic centimeters on the day before death. Studies of the blood were made as follows: 1. On admission: The hemoglobin content was 18.5 Gm.; the red cell count, 5,750,000; the white cell count, 29,300, with 95 per cent neutrophils and 5 per cent lymphocytes (1,465 per cubic millimeter). 2. The following day after 2,000 cc. of a 10 per cent solution of dextrose had been injected intravenously the hemoglobin content was 11.2 Gm.; the red cell count, 3,400,000; the white cell count, 22,600, with 94 per cent neutrophils, 1 per cent basophils and 5 per cent lymphocytes (1,130 per cubic millimeter). 3. Three days later and three days before he died the hemoglobin content was 10.5 Gm.; the red cell count, 3,800,000; the white cell count, 21,000, with 98 per cent neutrophils and 2 per cent lymphocytes (420 per cubic millimeter).

His temperature remained subnormal throughout his stay in the hospital. One thousand cubic centimeters of a 10 per cent solution of dextrose was injected intravenously twice daily. Caffeine and sodium benzoate injection U. S. P. failed to produce diuresis, and the patient died on the seventh hospital day with a terminal temperature of 96.2 F. The clinical diagnosis was poisoning due to ingestion of an unknown agent.

The necropsy diagnosis was: acute, hemorrhagic, necrotizing membranous gastroenterocolitis due to ingestion of an unknown poison, probably mercury; focal acute necrotizing stomatitis (mucous membrane of the cheeks); coagulative necrosis of the proximal convoluted tubules of the kidneys; focal pancreatic fat necrosis; edema and hemorrhage of the lungs.

The spleen weighed 120 Gm., and the malpighian bodies were indistinct. The thymic remains were inconspicuous. Peyer's patches and solitary follicles were obscured by the necrotizing change. The mesenteric lymph nodes were small and reddened, and the tracheobronchial lymph nodes were softened and succulent. All of these lymphatic tissues when examined histologically had a "generally washed out" appearance, and no active follicles were seen in any of them. In only one of the sections was anything suggestive of "reticular arrest" seen (fig. 2).

CASE 3 (severe third degree burns with bilateral symmetric cortical necrosis of the kidneys).—This case has been reported elsewhere[2]; hence it will be referred to only briefly. A white woman of 32 years died on the fifth day after severe third degree burns of thighs and trunk involving about two thirds of the total body surface. Despite forcing of fluids, she remained anuric, with the blood nonprotein nitrogen rising to 165 mg. per hundred cubic centimeters on the day of death. The only white blood cell count and differential count were made on the day of admission—white cells 3,300, with 87 per cent neutrophils, 11 per cent lymphocytes (363 per cubic millimeter), and 2 per cent monocytes. The spleen weighed 170 Gm. but was severely congested; microscopic study revealed small inactive malpighian bodies. Histologic examination of the lymph nodes dissected from the material fixed in Kaiserling solution and preserved at the time of necropsy showed the changes depicted in figure 3.

CASE 4 (lipoid nephrosis).—A white girl of 21 months entered the hospital on July 8, 1938 and died eleven days later. The complaints on admission were generalized edema (insidious in its onset and developing for three to four weeks), dyspnea and weakness. The clinical findings (extreme anasarca), the urinary findings (proteinuria [3 plus], a few casts and leukocytes and no red blood cells) and the results of chemical examination of the blood (urea nitrogen, 12 mg. per hundred cubic centimeters; blood plasma proteins, 2.9 per cent, with an albumin-globulin ratio of 1.4) all pointed to the diagnosis of lipoid nephrosis. On the day of admission the white cell count was 20,700, with 42 per cent lymphocytes (8,694 per cubic millimeter); one week later the count had fallen to 14,400, with 29 per cent lymphocytes (4,176 per cubic millimeter), and on the day before death the count was recorded as 4,250, with 21 per cent lymphocytes (893 per cubic millimeter). Permission for necropsy was not obtained.

2. Brown, C. E., and Crane, G. L.: J. A. M. A. **122**:871, 1943.

DUPLICATION OF THE VERMIFORM APPENDIX, THE LARGE INTESTINE AND THE URINARY BLADDER

REPORT OF A CASE

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Duplication, either partial or complete, of the vermiform appendix is a comparatively rare anomaly. Unless other grave abnormalities coexist, the condition is entirely compatible with a normal existence. The degree of duplicity varies widely, ranging from partial duplication to the presence of two distinct appendixes arising from a double cecum. The case reported in this paper reflects the latter degree of duplication and is interesting from the standpoint of the possible mode of genesis of the anomaly. In an 8 month old child, two appendixes had their origins from the two sides of conjoined ceca, which were almost equally divided by a septum that extended throughout the large intestine.

Less than 50 records of duplicity of the vermiform appendix have been found in the literature. The majority of these reports are brief clinical notes citing the presence of two appendixes encountered at surgical operation. The dearth of anatomic and histologic data in these reports makes it difficult to arrive at any conception of the embryologic origin of the anomalous appendixes.

In reviewing the published cases we have adopted, with minor modifications, the classification of Cave,[1] namely: (I) partial duplicity, (II) two appendixes arising from a single cecum and (III) two appendixes with a double cecum.

Five reported cases have been found in group I. Rosenberger[2] and Prentiss[3] each reported a widened organ consisting of conjoined appendixes with two separate lumens. Elwyn[4] described two appendixes which were distinct at their insertion but reunited at the tip. In Walmsley's example[5] the second appendix arose as a diverticulum of the main organ. The appendix found by Walthard[6] arose normally from the cecum, then formed two parallel tubes with a common serosa and longitudinal musculature, and these reunited distally to form a single appendix.

We have divided group II into two subgroups depending on whether the appendixes were found (A) at necropsy or (B) at surgical operation.

Six cases are recorded which fall into subgroup II A. The examples reported by Bartels,[7] Kermauner,[8] von Berenberg-Gossler[9] and Del Plaine[10] were associated with vesical-intestinal communication and incomplete development of the colon-rectum with atresia ani. Kermauner's case lacked histologic data. Emrys-Roberts and Paterson[11] found a small sacculated appendix on each side of the small intestine at its junction with the cecal pouch in a fetus, together with ectopia of viscera and spina bifida. Pratt[12] described a case in which two appendixes arose from a single cecum in a 15 day old white boy showing multiple anomalies.

Subgroup II B, containing the largest number of reported cases, has been further subdivided into three groups, namely: (1) cases of double appendix with description including anatomic and histologic data; (2) cases of double appendix with description including anatomic relationships but not histologic data, and (3) cases with brief description, lacking both anatomic and histologic data.

The 7 examples of group II B, type 1, were recorded by Leonardo,[13] Goldschmidt,[14] Bert-

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1. Cave, A. J. E.: J. Anat. **70**:283, 1936.
2. Rosenberger, R. C.: Proc. Path. Soc. Philadelphia **6**:207, 1902-1903.
3. Prentiss, E. C.: Washington M. Ann. **5**:25, 1906-1907.
4. Elwyn, A.: Anat. Rec. **27**:180, 1924.
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6. Walthard, B.: Deutsche Ztschr. f. Chir. **230**:413, 1931.
7. Bartels, M.: Inaug. Dissert., University of Berlin, 1867.
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9. von Berenberg-Gossler, H.: Anat. Hefte **49**:611, 1913.
10. Del Plaine, C. W.: Minnesota Med. **19**:736, 1936.
11. Emrys-Roberts, E., and Paterson, A. M.: J. Anat. & Physiol. **40**:332, 1905-1906.
12. Pratt, H. N.: Am. J. Dis. Child. **45**:1263, 1933.
13. Leonardo, R. M.: J. Internat. Coll. Surgeons **6**:64, 1943.
14. Goldschmidt, W.: Zentralbl. f. Chir. **57**:3123, 1930.

hold,[15] Clavel and Colson,[16] Piraja,[17] Cave [1] and Taylor.[18] The two appendixes were completely separate in the cases reported by Leonardo, Berthold, Cave and Taylor. The anomaly described by Goldschmidt is of doubtful authenticity. Piraja described a fixed cecum with the appendix originating at the usual site and, after a course of 6 cm., opening anew into the cecum. Cave obtained his specimen through the courtesy of H. C. Wilson, prosector of the Royal College of Surgeons' Museum. Taylor's example was found during a cesarean section.

Group II B, type 2, includes 6 instances of double appendix reported by Schooler,[19] Young,[20] Green,[21] Baz and Perrin,[22] Robertson [23] and Leonardo.[13] The reports of Schooler, Young and Green each included a description of 1 gangrenous appendix. The case reported by Robertson was described to him personally by Dr. Boulanger in 1936.

Group II B, type 3, contains cases reported by Fleischman,[24] Kaufman,[24] Braatz,[25] Adams-Ray [26] and Waugh,[27] all of whom recorded individual specimens. In addition, Bérard and Vignard [28] cited 2 examples, one belonging to Routier (1909) and the other to Jalaquier (1914).

The early literature pertaining to group III is vague. Original publications have not been available for a few early cases, including that of Delius and that of Rokitansky, described in 1846 and again in 1861.[29] Seven reports of double ceca and two separate appendixes have been found. Meckel's [30] example, reported in 1822, showed a double ascending colon; one limb terminated in a bulbous end (vermiform appendix?) and the other in a small diverticulum which might be interpreted as a second appendix. The duplicated appendix presented by Grohé [31] was encountered at operation in a 10 year old girl. The large intestine was divided by a septum which began in the region of the cecum; from the cecum originated two vermiform appendixes. Multiple anomalies of the external and internal genital organs, the rectum and the large intestine were present. Läwen [32] reported duplicate appendixes, duplicate ceca and a blind ending to the terminal part of the gut in a female child, who also had a meningocele. The anomaly cited by Tango,[33] occurring in a girl 1 year and 3 months of age, who died of dyspepsia, presented a double appendix and a double cecum, as well as incomplete development of the colon-rectum with atresia ani. Greig [34] recorded two appendixes occurring in a 10 week old girl with the whole bowel in duplicate distal to the site of a Meckel diverticulum. Each of the two separate ceca had its proper vermiform appendix. No histologic data were given in this report. Cave [1] described a pathologic specimen from the tetralogic series of the Hunterian Museum of the Royal College of Surgeons in England as an example of duplication of the appendix. In this specimen, obtained from a newborn infant, the last 17 cm. of the ileum, the cecum and the ascending colon were bifurcated. Mesially the ceca were adherent, and each cecum had a separate vermiform appendix. Likewise, Aitken [35] at autopsy on a girl 1 day old observed a septum extending from the lower part of the ileum to the rectum and completely dividing this part of the intestine into two halves. A small vermiform appendix measuring 2 cm. in length arose from the extremity of each cecum.

The case now reported is illustrative of group III.

REPORT OF CASE

A Negro boy 4 months of age was admitted to the Children's Hospital, Pittsburgh, on June 28, 1944 with a history of marked distention of the abdomen and fecal impaction. From the time of birth, evacuation of the bowel was irregular, and repeated enemas were necessary, but otherwise the child had no illnesses. He weighed 6 pounds and 10 ounces (3,005 Gm.) at birth and throughout life had been given a formula of cow's milk. The family history was irrelative, and there were no siblings. Before hospitalization, no bowel movement had occurred for three days, and several enemas and magnesia magma U. S. P. had been given without effect except to increase the abdominal distention. The child appeared physically well developed but somewhat undernourished. Physical examination showed no abnormality except abdominal distention, prominence of abdominal

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30. Meckel, J. F.: Tabulae anatomico-pathologicae, Leipzig, J. F. Gleditsch, 1822, no. 3, tab. 24, p. 14.
31. Grohé, B.: Deutsche Ztschr. f. Chir. **57**:445, 1900.
32. Läwen, A.: Beitr. z. path. Anat. u. z. allg. Path. **55**:575, 1913.
33. Tango, M.: Tr. Jap. Path. Soc. **19**:598, 1929.
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veins and absence of peristalsis. Rectal examination revealed an obstruction high up at the tip of the examining finger. The temperature fluctuated between 98 and 106 F. Preliminary diagnostic impressions were Hirschsprung's disease and fecal impaction. The child was treated with dietary changes, pitressin, oil retention enemas, castor oil, syntropan, mecholyl chloride and subcutaneous fluids, without improvement. A flat roentgenographic plate of the abdomen on August 10 showed a much dilated colon containing a great deal of gas and fecal material. The roentgenologic diagnosis was megacolon. A surgical consultation was held, but operative intervention was not deemed advisable. The child had a progressively downhill course and was sent home on September 25, with the family realizing the poor prognosis. Death occurred at home on Oct. 28, at 9 a. m. The mother requested an autopsy on the child, whose body was brought back to the hospital.

Autopsy (seven and one-half hours after death).—The body was that of an emaciated Negro boy 8 months of age and 45 cm. in length. Postmortem rigidity was absent, and postmortem lividity was marked in the dependent parts. About 50 cc. of foul-smelling watery fluid escaped from the nares and the mouth. The thorax appeared small in proportion to the greatly distended abdomen. The skin over the abdomen was so tense that it was thinned like parchment, and irregular patches appeared leukodermic, while other areas showed greenish-blue discoloration. On rectal examination, the walls of only one cavity could be palpated, and no evidence of an anal septum was encountered by the examining finger.

The subcutaneous tissue was devoid of fat. The abdominal wall lacked muscle except in irregular patches and was composed almost entirely of the thinned skin. The thoracic cavity was markedly decreased by the domelike diaphragm, which had been pushed upward by intra-abdominal pressure. The thymus was small and appeared normal. The heart was displaced toward the right side of the chest, and the descending aorta was situated on the right side of the vertebral column, occasioning a stretching of several of the left vertebral arteries. The esophagus was located on the left side of the aorta, almost directly overlying the vertebral column. The pericardium and the heart appeared normal. The right lung was slightly compressed by the overlying heart, and both lungs showed considerable congestion.

The colon was markedly dilated. Incomplete rotation of the gut was evidenced by the median position of the cecum and by lack of peritoneal fixation to the right lateral abdominal wall. The peritoneal fixation on the left side of the abdomen was normal. The markedly thinned great omentum was normal in position. The cecum was lying free on the left side at the level of the umbilicus on a plane anterior to the small intestine and the superior mesenteric artery. The ileum entered the cecum from right to left. The rotation of the gut had stopped at about the middle of the second stage [36] or approximately the tenth week of prenatal life. The mesentery appeared normal except for a shallow accessory fold (fig. 1), 16 cm. long, in the region of the ileocecal valve. The entire large intestine was divided longitudinally by a congested central groove.

Two separate vermiform appendixes (figs. 1 and 2) measuring 5.8 by 0.7 and 6.3 by 0.8 cm., respectively, arose 1.8 cm. apart on the two sides of the cecal groove. In the proximal third of their course they had a common mesoappendix containing a small lymph node, and both were supplied by the same artery, which bifurcated 1 cm. distant from their insertion. The large intestine was removed without opening about 2 inches above the anal orifice, fixed in Jores solution [36a] and after being photographed was sectioned. The viscus, which was engorged with gas and feces, was completely separated into two unequal parts by a septum which began at the ileocecal valve and extended almost to the anus. The less dilated portion of the colon, with an average diameter of 2.5 cm., had a rectal opening and connected with the ileum through the ileocecal valve which measured 1.1 cm. in diameter. The wall of this portion of the intestine was of normal thickness. The only entrance into the more dilated part of the colon was a small oval aperture in the septum, measuring 0.8 by 0.6 cm., situated immediately above the ileocecal valve. The wall of this cavity was exceedingly thin. This part of the colon ended blindly in the rectum or had a small opening into the patent half. Only two taeniae coli could be found. Each originated at the base of one of the appendixes and extended along the free lateral surfaces.

The spleen, located on the right side of the body beneath the lobus caudatus of the liver, showed nothing unusual. The pancreas was a soft lobulated organ with the head in the usual position in relation to the duodenum and the tail of the organ on the right side of the body in relation to the spleen. The liver was of normal size and showed mild chronic passive congestion and fatty infiltration. The gallbladder and the adrenal glands appeared normal. The renal pelves were distended, and both ureters were dilated down to the promontory of the sacrum. On removal of the thin-walled bladder, a septum was found extending anteroposteriorly, dividing the organ into two halves. A ureter entered the bladder on each side of the septum.

The anatomic diagnosis was: duplication of the vermiform appendix; duplication of the large intestine; bifurcating septum of the colon and the rectum; incomplete rotation of the gut; dilatation of the large intestine; congenital absence of one taenia coli; anomalous position of the taeniae coli; bifurcating septum of the bladder; elongation of the vertebral arteries (seventh, eighth and ninth) on the left side; chronic passive congestion of the liver and the lungs; displacement of the heart, the descending aorta and the spleen (to the right); dilatation of the ureters.

Microscopically, the lungs showed marked chronic passive congestion, interstitial pneumonia, many areas of emphysema and a few patches of atelectasis. The liver was irregularly congested, probably owing to the effect of the increased intra-abdominal pressure, and showed granular degeneration and serous hepatitis. The kidneys showed acute interstitial nephritis. All stages of retrograde changes from early granular degeneration to necrosis could be followed in the proximal convoluted tubules. The glomeruli were numerous and enlarged, and many contained vacuolated and pyknotic nuclei. The cause of the acute interstitial nephritis was not clear, as evidence of fulminating infection was not found. This condition may possibly have been due to pressure from the distended intestine associated with absorption of intestinal toxins. The wall of the bladder had a normal appearance and the septum of the bladder showed a doubling of normal parietal structures. In the large intestine the septum separating the two lumens showed all the components of the normal intestine

36. Dott, N.: Brit. J. Surg. **2**:251, 1924.

36a. Mallory, F. B.: Pathologic Technique, Philadelphia, W. B. Saunders Company, 1938, p. 381.

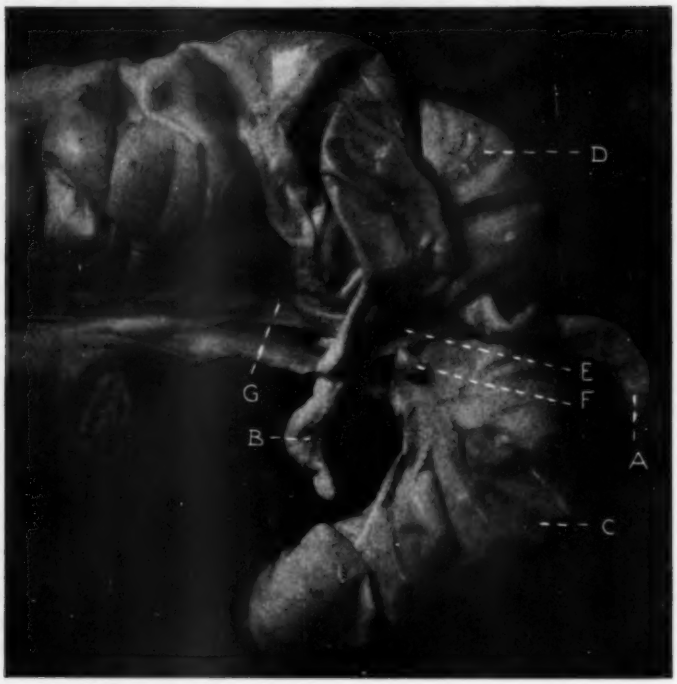

Fig. 1.—Terminal part of the ileum and the cecum showing the origin of the two appendixes: *A* and *B*, appendixes; *C*, ileum; *D*, less dilated portion of the colon; *E*, common mesoappendix; *F*, lymph node; G, secondary mesenteric fold. Natural size.

Fig. 2.—Duplicated large intestine opened along the dividing septum. *A* and *B*, appendixes; *C*, ileum; D, rod through an oval aperture in the septum; *E* and *K*, rods in the lumens of the appendixes; *F*, lymph node; *G*, septum; *H*, rod in the ileocecal valve; *I*, less dilated portion of the colon; *J*, greatly dilated portion of the colon. Natural size.

in duplicity except a somewhat thinned adventitia. The walls of both lumens of the large intestine contained throughout both the circular and the longitudinal layer of muscle. Each of the two appendixes showed a normal structure, including circular and longitudinal muscular layers, as well as a normal amount of lymphoid tissue.

COMMENT

The human appendix is a distinctive organ with a definite structure which is possessed only by man, the gibbons and the anthropoid apes. A characteristic feature of this organ in these forms is the lymphoid and muscular tissues. The lymphoid tissue is moderate in amount at birth, after which it develops gradually, reaches a maximum in the adult and declines in old age.[37] The circular and the longitudinal musculature are well developed. Any theories advocated to explain the duplicity of the vermiform appendix, to be acceptable, must take into account this anatomic structure. The earlier theories advanced to explain duplication of the vermiform appendix are related to the persistence of vestigial structures associated with the normal ontogenetic development of the cecum.

Mitchell[38] postulated that the human vermiform appendix represents persistence of one of the original paired avian ceca and suggested that the occasional appearance of a double appendix is a phylogenetic reversion. This theory is negated by the fact that the bird is not a direct ancestor of man, but rather a relative.

Jones[39] similarly argued that the primitive promammalian cecum was a paired lacertilian structure and that the greater specialization of the large intestine in the mammalians led to the ultimate obliteration of one of the ceca, with the remaining half persisting as a single human vermiform appendix. An anomalous condition with the presence of two appendixes indicated persistence of the primitive paired structures. This genesis is possible but not probable. In those cases in which histologic data are available, there is no evidence that one is dealing with a primitive structure.

Another explanation of the occasional existence of two appendixes is based on the persistence and development of a transient appendix, first described by Kelly and Hurdon,[40] in 1905, after a study of over 50 embryos in the Mall and Brodel collection. This fugitive appendix consists of an outgrowth from the tip of the developing cecum which enlarges during the sixth week of prenatal life and atrophies toward the end of the seventh week. Because it atrophies and disappears early, it is considered an independent structure, not directly related to the permanent vermiform appendix which appears later.

Recently Bremer[41] has suggested a new theory of the genesis of intestinal anomalies, which he divided into two groups according to their origin: diverticula and duplications. The diverticula arise through persistence and further development of minute epithelial buds, which Keibel[42] and Lewis and Thyng[43] have described as occurring in the epithelial lining of the gut in the fetus of 8 to 9 weeks of age. Normally these buds disappear. When they persist, they extend into the mucosa or the serosa and on subsequent expansion form giant diverticula and cysts, the walls of which are characterized by a lack of lymphoid tissue and circular and longitudinal musculature.

Bremer's theory of the genesis of duplications is based on the observations of Johnson[44] and of Fischer[45] regarding the development of vacuoles in the proliferating lining epithelium of the small gut in the fetus of 6 weeks of age or 10 mm. of length. The hyperplastic epithelium forms at irregular intervals along the course of the lengthening gut solid cords, the cells of which secrete centrally drops of mucus and then flatten out and disappear. This secretion is related to the formation of the intestinal lumen. Occasionally the drops of mucus remain in the epithelial wall and where they coalesce become alined longitudinally to form supernumerary tubes parallel to the main mesial tube. On development of musculature the supernumery tubes form duplicate intestines parallel to the main intestine. Bremer has pictured diagrammatically the development of a progressive series of these duplications. Both Lewis[46] and Johnson[47] stated that the vacuoles are less conspicuous in the large intestine, where they develop at a later period, than in the small intestine.

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39. Jones, F. W.: J. Anat. & Physiol. **46**:193, 1912.

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47. Johnson, F. P.: Am. J. Anat. **10**:521, 1910.

The secondary viscus may attain such size and importance as to be practically equal to the original, as in our anomaly, and this suggests that the whole process may fundamentally be a division of the forces or the substances responsible for the determination of the part of the intestinal tract involved.

The anomalous morphogenesis, which in our case terminated in a duplication of the large gut, the vermiform appendix and the urinary bladder, must have been initiated before the separation of the latter organ from the cloaca by the urorectal septum. This separation occurs early—according to Lewis,[46] in embryos between 4.9 mm. nape length and 5.3 mm. greatest length. The genesis of our anomaly thus antedates the formation of mucogenic epithelial nodules, whose first appearance Bremer ascribed to the embryo of 10 mm. Furthermore, the epithelial nodules forming the solid stage occur mainly in the small gut and are inconspicuous or lacking in the large gut. The mucoid vacuolation of cells in the primitive foregut may be the modus operandi in the formation of certain intestinal duplications, but the separation of the bladder into two equal halves is not mediated by the formation of epithelial vacuoles. We believe that the explanation of twin formation of the whole of the primitive large gut is more intimately associated with those embryonic induction forces which progressively determine morphogenesis and histogenesis rather than with vacuole formation, known to be inconspicuous in the large gut.

We are of the opinion that a more adequate explanation is offered by the organizer concept for morphogenesis promulgated by Spemann[48] and Needham[49] as a result of experiments in embryology. Homeotransplantation of fragments of blastopore lip as well as other tissues into the lip of the blastopore resulted in the formation of new morphologic entities in addition to those which would have developed normally. Depending on the age of the blastopore at the time of transplantation, a wide range of anomalies developed, culminating under optimal conditions in twin formation. The substance inducing the change was termed the inductor or organizer and the area acted on the individuation or induction field. Embryonic morphogenesis is conceived as a progressive series of coordinated growth and differentiation phases, each of which is dependent on and induced by substances elaborated by the tissue differentiated in their immediate sphere of activity. A classic example of regional "organizer" morphogenesis is the successive development of pronephros, mesonephros and metanephros. Qualitative and quantitative variations in the organizer can modify the type and the extent of the resultant regional morphogenesis. Depending on the state of the embryonic development at which modifications or alterations in the stimulus react and on the competence of the induction field, a progressive series of anomalies may occur. The artificial production of developmental anomalies by subjecting the developing embryo to such varied stimuli as magnesium chloride and insufficient oxidation (Stockard[50]), ultraviolet radiation (Hinrichs and Genther[51]), neutron bombardment (Enzmann and Haskins[52]) and increased temperature (Harman[53]) has been interpreted as due to interference with the organizer. It seems plausible that extraneous factors may similarly act in the human embryo to modify the normal formative "organizer" effect so that developing plastic tissue may undergo pathologic differentiation. In the case of the vermiform appendix, these duplications may range in type from a double-barreled appendix to complete division of the entire large intestine with associated double appendixes and varying defects of the urovesical system. If the appendix only is duplicated, the interference involves only the organizer acting on the appendical anlage; if there is duplication of the entire large intestine with associated doubling of the appendix and the bladder, the disturbance in organizer must occur in the anlage of the large intestine before the separation of the gut from the urovesical tissue.

SUMMARY

The occurrence of a double bladder and a double large intestine together with two separate vermiform appendixes indicates that the anomalous morphogenesis was initiated before the separation of the urorectal septum. This separation begins in an embryo of 5.3 mm. greatest length, which is much earlier than the formation of mucoid vesicles recently postulated as the method of origin of intestinal duplications. An alternate theory is that a disturbance in organizer may be the origin for such anomalies.

48. Spemann, H.: Embryonic Development and Induction, New Haven, Conn., Yale University Press, 1938.

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50. Stockard, C. R.: Am. J. Anat. **28**:115, 1921.

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CANCER IN RELATION TO USAGES

THREE NEW TYPES IN INDIA

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Les causes [de chancre] sont deux, antécédente et coniointe.—Ambroise Paré.[1]

Several types of human cancer have been traced to the action of substances to which people are exposed during the course of their everyday life. Some of these are associated with occupational hazards, whereas others are related to the habits of the people. During the last five years we had occasion to study in different parts of India three types of cancer, which, on investigation, we believe may be attributed to certain usages prevalent in those regions.

DHOTI CANCER

Among the first 5,000 patients who attended the clinics of the Tata Memorial Hospital, in Bombay, there were 3,003 with carcinoma. The lesion was located in the mouth and the oropharynx in the majority of cases. Carcinoma of the skin was relatively rare, as it was seen in only 63 persons, the two types (squamous cell and basal cell) being equally represented. It may be seen from table 1 that carcinoma of the skin is more in evidence among non-Indians than among Indians and, further, that the basal cell variety is much more so. The latter type was proportionally more common in non-Indians (11 per cent) than in Indians (0.7 per cent).

Carcinoma of the skin of the trunk was traceable only among Indians, and its incidence was found to be much higher among them than among white peoples. Schrek[2] in an analysis of 2,580 cases of cutaneous tumors, based on the records of the Pondville Hospital, Wrentham, Mass., and the Massachusetts Tumor Diagnosis Service, noticed that the large surface of the trunk (excluding the external genitalia and the anal orifice) was the site of carcinoma in relatively few cases (4.7 per cent) and that tumors of this class were mostly basal cell in type, the cases of basal cell carcinoma being 68 per cent of the total. Rose[3] in his thesis remarked that the skin of the trunk was rarely the seat of primary epithelioma (excluding melanoma) and that until 1920 only 84 cases had been reported in the literature on the subject. In the material at the Tata Memorial Hospital, there were 23 cases of squamous cell carcinoma of the skin, and in 8 the lesion was situated on the trunk of a male, and in 1, on that of a female. There was 1 case of basal cell carcinoma of the trunk, the patient having multiple tumors of this type on the face and the body. The cases of squamous cell carcinoma of the trunk were distributed as follows: loin, 2; groin, 5; buttock, 1; rump, 1.

TABLE 1.—*The Incidence of Carcinoma of the Skin*

	Indians		Non-Indians	
Total attendance at the clinics of Tata Memorial Hospital, in Bombay	Males 2,985	Females 1,655	Males 203	Females 267
Total number with carcinoma	1,946	903	71	53
Number with carcinoma of the skin....	36 (1.85)*	6 (0.64)	15 (21.13)	5 (9.43)
Number with squamous cell carcinoma	20	3	6	0
Number with basal cell carcinoma	16	3	9	5

* The figures in parentheses represent percentages of cutaneous carcinoma relative to total carcinoma.

Two illustrative cases of squamous cell carcinoma of the trunk are recorded here, and the findings in all the 9 cases are summarized in table 2.

CASE 2.—A Hindu farmer of Deccan, aged 62, presented himself with an ulcerated tumor (4 by 6 cm.) situated above the right iliac crest (fig. 1 *A*). The nonulcerated portion of the tumor was papillary in character. The edges of the ulcer were hard and everted. The tumor was fixed to the subjacent tissues. The skin surrounding it was edematous and showed scarred, depigmented patches below the level of the ulcer in its immediate vicinity. The skin of the corresponding area on the opposite loin was dusky, coarsely corrugated, with a thin, smooth patch of depigmentation in the middle. There was a hard projecting fixed swelling, the size of a hen's egg (5 by 3 by 3 cm.), in the right

From the Pathology Departments of Tata Memorial Hospital, Bombay, and Andhra Medical College, Vizagapatam.

1. Paré, A.: Oeuvres complètes, edited by J. F. Malgaigne, Paris, J.-B. Baillière, 1840, vol. 1, book 5, chap. 25, p. 362.

2. Schrek, R.: Arch. Path. **31**:411, 1941.

3. Rose, K.: Ueber Rumpfhautkarzinome, Breslau, 1920; cited by Beck.[10]

inguinal region. The scrotal skin was eczematous in some places and showed several sebaceous cysts. Careful clinical examination, laboratory investigations and screening for diseases of the chest did not reveal any other abnormalities except a fasting blood sugar level of 160 mg. per hundred cubic centimeters and mild polymorphonuclear leukocytosis. The Kahn test of the blood was negative, and there were no stigmas suggestive of syphilitic infection.

The patient stated that the lesion had started as a small ulcer in the right loin twelve months previously and had gradually increased in extent. He had suffered from a similar small ulcer in the same region about fifteen years earlier and from another ulcer on the opposite side a little later. These ulcers had healed after application of leeches and other local treatment. He admitted that he always wore his dhoti tight, so that it exerted continuous pressure on the two loins. Both sides showed scars of healed ulcers.

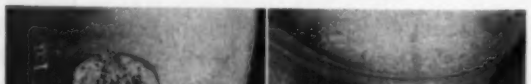

Fig. 1.—*A*, trunk of a wearer of the dhoti with an ovoid cancerous ulceration above the right iliac crest and a depigmented patch of skin just below it. The inguinal mass of lymph nodes, site of metastasis of a dhoti cancer, is well seen in *B*.

The tumor and a wide area of skin around it were excised with a thermocautery. The excised area healed with grafting of skin; the inguinal region was treated with high voltage roentgen radiation (1,000 roentgens [r], 200 kilovolts, 15 milliamperes, 10 cm. field; target skin distance, 50 cm.). A month later the mass in the groin was removed by radical dissection of the groin. The tumor tissue was necrotic and adherent to the femoral vessels. Within one month a lymph node in the right axilla became enlarged, and was treated with radiation (axillary portal, 7.5 cm.; total dose, 2,000 r). The patient died at his home one month later. No autopsy was possible.

The histologic examination of the tumor showed the characters of a squamous cell carcinoma, grade 2. The depigmented skin showed hyperkeratosis, increase of prickle cells, depigmentation of the basal layer and flattening of the papillae. The mass in the inguinal region and the nodule in the axilla evidenced metastatic involvement of the lymph nodes.

Case 5.—A Hindu man of Deccan aged 72 was admitted to the hospital with a deep ulcer in the groove of the right groin (fig. 1 *B*). The ulcer was 5 cm. long, with a hard roll of everted tissue on the lateral side and a scraggy raw area extending onto the skin of the scrotum on the inner side. The floor of the ulcer was fixed to the deeper tissues and was covered with unhealthy granulations, alive with maggots in it. The adjacent scrotal tissue, the penis and the perianal tissue showed no evidence of any morbid condition. The general condition of the patient was good, and the clinical and the laboratory findings were normal except for moderate leukocytosis. The Kahn test was negative. The blood sugar amounted to 105.2 mg. and the serum nonprotein nitrogen to 18.7 mg. per hundred cubic centimeters.

The patient stated that he had noted a "boil" in the groin three years previously. It slowly increased in size and burst four months later. He was being treated by his doctor with courses of "injections" and local applications, without any improvement. His history revealed that he always wore his dhoti with the "perineal fold" on the right side and had always kept his dhoti on his body while bathing. Biopsy of the tumor showed it to be squamous carcinoma, grade 2, with superficial necrosis and ulceration. The tumor was widely excised with a galvanocautery, It slowly healed completely after two attempts at skin grafting. The disease seemed to have been well eradicated; there has not been any recurrence or complication during the fourteen months of follow-up observation.

A study of the cases listed in table 2 revealed that in every one of them the tumor occurred in a person who wore a dhoti or a saree and that the location was along the lines of the greatest friction or pressure of the garment. It may be necessary to describe briefly the method of wearing a dhoti, as many persons may be unacquainted with the manners and customs of the people in India.

The dhoti is a piece of wide cotton cloth usually 5 meters long and 1.5 meters broad (about 16½ feet long and 5 feet wide). It is worn as a nether garment to cover the lower part of the body by being wrapped around the trunk

Table 2.—*Summary of the Findings in Cases of Dhoti Cancer*

Case	Sex	Age, Yr.	Community	Lesion	Histologic Diagnosis
1	F	35	Hindu Deccani	Ulcer on left loin; patches of depigmentation on right loin	Squamous cell carcinoma, grade 2
2	M	62	Hindu Deccani	Ulcer above iliac crest in right loin (fig. 1*A*)	Squamous cell carcinoma, grade 2
3	M	40	Muslim	Tumor projecting from skin of inner end of right groin	Papillary squamous cell carcinoma, grade 1
4	M	50	Hindu Deccani	Fungating ulcer of right groin	Squamous cell carcinoma, grade 1
5	M	72	Hindu Deccani	Fungating ulcer involving skin of right groin and extending over to surface of scrotum	Squamous cell carcinoma, grade 2
6	M	50	Hindu Deccani	Ulcerated growth in fold of left groin	Epidermal carcinoma, grade 2
7	M	65	Hindu	Fungating ulcer of right groin	Squamous cell carcinoma, grade 2
8	M	32	Hindu Deccani	Warty growth on inner side of right buttock	Malignant ? Papilloma of skin
9	M	70	Hindu Deccani	Fungating tumor mass behind sacrum	Squamous cell carcinoma, grade 2

and the lower extremities. The method of wearing it which will be described is the one obtaining among Deccani Hindus, who form the bulk of the laboring class in the southern part of the Bombay Presidency. The Gujrati Hindus do not wear the dhoti so tightly, and the free edge is crossed along the left groin. Poorer Muslims also wear a dhoti in many parts of India. The women wear a similar garment known as the saree (or sari), but the method of wearing it is different, the points of pressure being in the two loins. The dhoti is usually worn by holding the upper edge behind the small of the back, with the shorter one fifth in the left hand and the longer four fifths in the right hand. The two pieces are brought forward until they cross over in front for a short distance. The crossed portions are tightened, rolled and made secure at the waist in order to hold the garment in place. The free edge of the shorter piece is taken along the right groin to the back and tucked in the upper edge behind the

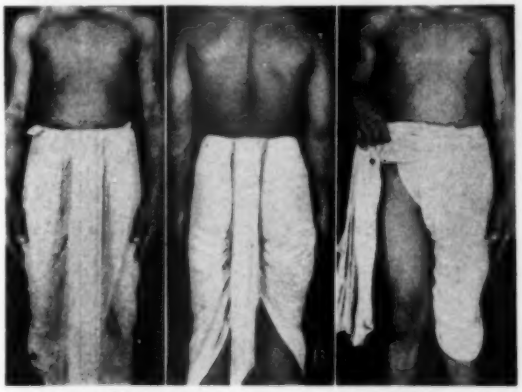

Fig. 2.—The method of wearing a dhoti. In *C* the pleats of the free portion of the longer piece are pulled to the right to show the perineal fold of the dhoti in front.

sacrum. This may be termed the perineal fold of the dhoti. The free portion of the longer piece is folded in pleats and tucked in front below the navel (fig. 2). It is evident from the foregoing description that the dhoti exerts constant pressure in the two loins, the right groin, the inner surface of the right buttock and along the midline behind the sacrum. Most of the wearers work and sleep in the same garment and, because of a lack of closed washing places, clean and bathe themselves with a dhoti or a saree on the body. Because of this the sites of the body subjected to the greatest friction by the garment are often soiled with dust and sweat and have the least opportunity of a thorough cleansing.

The occurrence of cancer at the sites mentioned induced us to examine the loins and the groins of healthy persons of the poorer classes. It was observed that these regions were often the seat of cutaneous involvements, such as ringworm, scabies and chronic eczema. Even in the absence of any parasitic or bacterial lesions it was noticed that the skin above the iliac crests and in the groins was darker, coarser and slightly corrugated. In some persons the central portion of the altered, shagreen-like skin was depigmented, thin and glazed. The condition had been present for several years without any obvious discomfort. Biopsy specimens removed from these areas are represented here by a piece of skin from the loin of an Indian (fig. 3 *A*). Such specimens show histologically a covering of stratified squamous epithelial cells, with a thin layer of keratinized scales on the surface. The rete cones are widened and irregular. The basal layer of cells contains many coarse pigment granules. The papillary layer of the dermis is thin and rarely exceeds the thickness of the epithelial layer. It is made up of loosely packed fine collagen fibrils, few elastic fibers, thin-walled blood capillaries and lymph spaces. Histiocytes and lymphocytes are sparsely scattered in the vicinity of the vascular channels. The reticular layer of the dermis is five to six times thicker than the papillary layer and is composed of coarse intersecting strands of collagen, richly interspersed with dense elastic fibers. The tissue appears to be regular and evenly disposed in layers. It is traversed by many coiled ducts of sweat glands. Hardly any hair follicles or sebaceous glands are seen in the longitudinal sections.

Sections of skin taken in the same region, and similarly orientated, from persons who wear the dhoti usually show an increase in the superficial layer of the dermis with papilliferous corrugation of the surface epithelium (fig. 3 *B*). The rete cones are flattened and irregular. The basal layer of epithelial cells contains an increased amount of coarser granules of pigment. The reticular zone shows an irregular disposition of fibrous and elastic tissue, the elastic fibers being fused in coarse clumps or frayed in irregular strands between an irregular network of collagen tissue. The histologic observations in cases in which the skin is altered to a dusky corrugated appearance (fig. 3 *C*) with areas of depigmentation in the center are somewhat striking. The epithelial layer is thickened and hyperkeratotic in the darker area. The rete cones are widened, deepened and branched. The malpighian cells in the epithelium appear swollen and are covered by a dense, thick keratinous layer. The papillary layer of the dermis is increased several fold in depth. The whole thickness is occupied by loosely packed fine nonrefractile fibers of collagen, traversed vertically by conspicu-

ous parallel coiled and dilated blood capillaries (fig. 3 *D*). In this layer there is no cellular exudate indicative of a chronic inflammatory process. The recticular layer is denser than normal but does not show any marked alteration in structure. In the paler depigmented areas the epidermis is thinned out and flattened. The papillary layer of the dermis is not noticeable, and the collagen bundles are thickened and dense. Blood vessels, lymph spaces and coil glands are obliterated. A dense, compact tissue directly underlying the epithelial layer is formed in places and appears to be different from the fibrous tissues in its tinctorial affinities (fig. 3 *E*). It assumes a homogeneous orange color with Masson's trichrome stain and reddish brown with

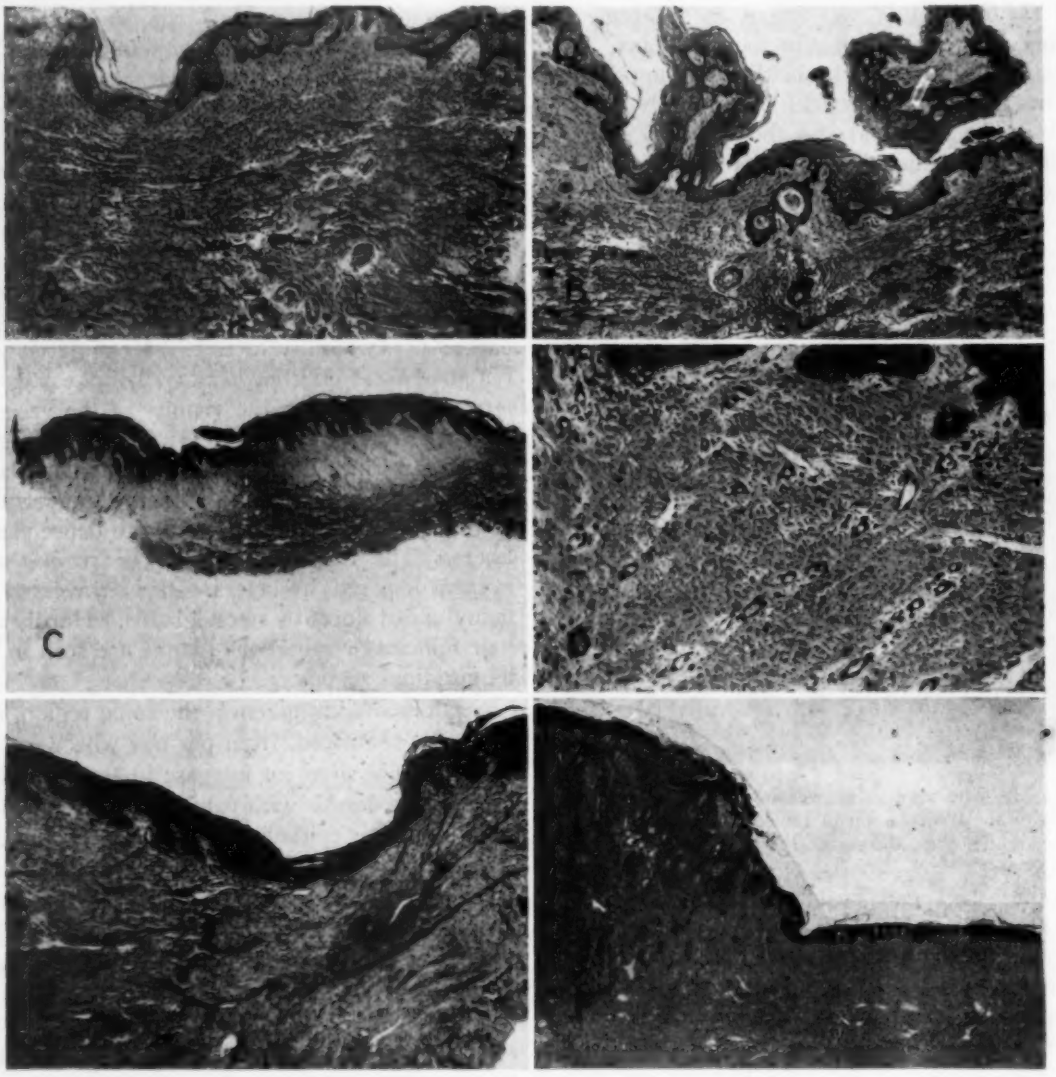

Fig. 3.—*A*, section of skin from the loin of a healthy Indian (paraffin embedding orcein-trinitrophenol-nuclear-fast red preparation; low power [× 47] photomicrograph). Note the slight irregularity in the epidermis, the thin papillary layer and the broad reticular layer of the epidermis.

B, section of skin from the loin of a person habitually wearing a dhoti (same region as shown in *A*; same method of preparation and magnification). Note the papilliferous corrugations of the surface epithelium and the broadening of the papillary layer of the dermis.

C, altered skin from the loin of a person wearing a dhoti; × 10; Leitz Elmar; 5 cm. (paraffin embedding; Masson trichrome stain). Note the acanthosis and the widening, deepening and branching of the rete cones. There are three clear areas of the markedly deepened papillary layer of the dermis traversed by parallel coiled blood capillaries. In two places the reticular layer is denser and almost adherent to the epidermis.

D, the clear portion of the papillary dermis in *C* showing the parallel coiled dilated blood capillaries embedded in loosely packed fine collagen material; × 103.

E, dense avascular tissue directly adherent to the epidermis; × 47.

F, dhoti cancer (left) and adjacent normal skin; × 6; Leitz Elmar; 5 cm. (paraffin embedding; hematoxylin and eosin stain.)

Van Gieson's stain. The histologic appearances suggest a marked disturbance of circulation in the dermis and consequent alteration of metabolism in the overlying epithelial cells. These appearances are reminiscent of the changes which Kreyberg [4] observed in the skin of mice after painting with tar, and Orr [5] after applying methylcholanthrene. The histologic changes which result in the development of a squamous cell carcinoma as seen in human material need no description except to state that the phenomena of proliferation and the supervention of secondary infection obscure the early changes which supply the basis for the development of cancer (fig. 3 *F*).

CHUTTA CANCER

In Vizagapatam and the outlying districts of Andhra Province, in the mideastern part of India, carcinoma of the hard palate appears to be fairly common. Kini and Subba Rao [6] in a clinical study of 335 cases of oral carcinoma found the lesion located on the palate in 52 cases. This type of cancer has been regarded by clinicians in this region as due to irritation from the habit of smoking a local type of cigar, called the chutta, with its burning end inside the mouth. The chutta is a roll of dried tobacco leaf ,which is made into a small cigar and tied at one end with a piece of thin string. Although the tobacco is strong, the chutta is a poor substitute for a cigar, as the smoke cannot be easily pulled, owing to the irregular core. The lighted end repeatedly goes out, and poorer folk resort to the habit of holding the lighted end inside the oral cavity to keep it burning for a longer time. This *Adda Poga,* or reverse smoking, is a habit which is frequently practiced in that part of the country by both men and women. The lighted cigar often goes round the family, and even children are allowed to have an occasional whiff at it.

It is seen from the hospital records in this region that leukoplakic lesions of the hard palate are frequently observed among chutta smokers. A cancerous process commences in an area of altered, opaque epithelium or more frequently starts as a small ulcer or a button-like nodule in a deeply congested, sore patch of mucous membrane. The leukoplakic condition may spread and involve the whole palate before the supervention of a cancerous process. The soft palate is rarely the seat of origin of carcinoma, although it may occasionally be involved in a spreading cancerous lesion. A lesion extending backward to the pillars of the fauces and the oropharynx is rare, though the gums on the upper jaw are often involved in the spread of the disease. Perforation of the hard palate with the process extending to the maxillary antrum is common. An early metastatic involvement of the submaxillary and upper deep cervical lymph nodes considerably worsens the prognosis. Death usually results from inanition, owing to marked difficulty in swallowing any type of food, or from aspiration pneumonia consequent on an extensive ulcerative lesion. The histologic examination of the tumor tissue usually reveals keratinizing squamous cell carcinoma with or without attendant leukoplakia of the adjacent mucous mem-

Fig. 4.—Chutta cancer: *A*, case 1; *B*, case 2.

4. Kreyberg, L.: Virchows Arch. f. path. Anat. **273**:367, 1929.

5. Orr, J. W.: J. Path. & Bact. **46**:495, 1938.

6. Kini, M. G., and Subba Rao, K. V.: Indian M. Gaz. **72**:677, 1937.

brane. Occasionally the tumor presents marked anaplastic cell characters, with the cells assuming a spheroid shape and leading to a mistaken diagnosis of sarcoma.

A brief clinical report of two representative cases of chutta cancer may be of interest.

1. A Hindu woman 55 years old was admitted with a history of difficulty in swallowing and pain in the gums. The patient complained of bleeding from the gums and ulceration of the palate. She admitted that she had been in the habit of smoking a chutta with the lighted end inside her mouth for the three previous years. There was extensive leukoplakia of the palate (fig. 4 *A*) with scattered small areas of superficial ulceration. The Wassermann reaction of the blood was negative, and there was no clinical evidence of syphilitic infection. The gums on the upper alveolus were soft and tender and bled easily. The lymph nodes in the submaxillary region and in the neck were not enlarged.

Biopsy of the edge of one of the ulcers on the palate showed leukoplakia of the mucous lining, with histologic appearances suggestive of an early cancerous transformation.

2. A poorly nourished, anemic Hindu woman, mother of 6 children, was examined because of pain in the hard palate and difficulty in swallowing. She stated that her complaint had started with redness and pain some time previously. On examination a swelling was observed in the right maxillary region which extended to the side of the nose (fig. 4 *B*). The skin over the swelling was glossy and hyperemic. Inside the oral cavity was an ulcer involving the whole of the hard palate and extending into the maxillary antrum and the right nasal cavity. The edges of the ulcer were raised. All the teeth on that side of the upper jaw were loose. A small perforation through the base of the ulcer communicated with the maxillary antrum.

An analysis of biopsy records of the department of pathology of Andhra Medical College, Vizagapatam, gives an idea of the relative incidence of palatal cancer as compared with cancer of any other site inside the oral cavity (fig. 5). where there is no smoking of chuttas, the relative proportion of carcinoma of the palate to that of the tongue was 6.2: 52.2, whereas in Vizagapatam palatal carcinoma was almost six times as frequent as carcinoma of the tongue.

KHAINI CANCER

It had been previously observed [7] that "cancer of the lip accounts for nearly 20 per cent of the oral cancer at the Memorial Hospital, in New York, and only 1.7 per cent at the Tata Memorial Hospital, in Bombay." During a recent tour in Bihar (northeastern part of India) we were, however, informed, much to our surprise, that cancer of the lower lip was the commonest type of oral cancer seen in the outpatient clinics of the

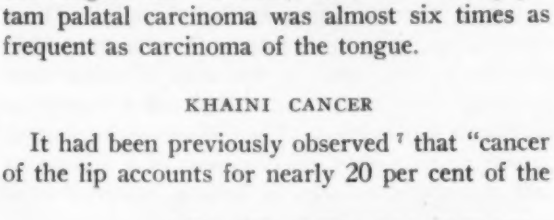

Fig. 5.—Distribution of intraoral cancer according to sites.

TABLE 3.—*Distribution of Intraoral Cancer According to Sites*

	Bombay, India, Tata Memorial Hospital, 1941-1943			Patna, Bihar, India, Prince of Wales Medical College, (Biopsies) 1934-1943			Vizagapatam, India, Andhra Medical College (Biopsies)		
	Males	Females	Total	Males	Females	Total	Males	Females	Total
Lip	10	7	17 (1.7)*	17	1	18 (12.6)	14	6	20 (7.0)
Buccal mucosa	111	54	165 (16.5)	35	5	40 (28.0)	30	14	44 (15.4)
Floor of mouth	19	4	23 (2.3)	3	1	4 (2.8)	3	3	6 (2.1)
Alveolus	24	13	37 (3.7)	25	5	30 (21.0)	9	5	14 (4.9)
Tongue	479	43	522 (52.2)	19	7	26 (18.2)	55	24	79 (27.7)
Tonsil	159	15	174 (17.4)	7	2	9 (6.2)	12	5	17 (6.0)
Palate	56	6	62 (6.2)	15	1	16 (11.2)	65	40	105 (36.8)
Total	858	142	1,000	121	22	143	188	97	285

* The figures in parentheses represent percentages of oral cancer relative to total cancer.

It is seen that the hard palate stands highest in the list of sites of oral carcinoma. Thus, among 285 cases of oral carcinoma there were 105 cases of carcinoma of the palate. This is in striking contrast with the incidence according to oral sites on the opposite coast of India. In Bombay, Medical College Hospital at Patna. A study of the biopsy records of the department of pathology of the Prince of Wales Medical College, Patna, during the ten year period from 1934 to 1943 (table 3) showed that there were 18 specimens

7. Khanolkar, V. R.: Cancer Research **4**:313, 1944.

from lip cancer among 143 tissues sent for histologic examination in cases of oral carcinoma. It was, however, remarked that patients with labial carcinoma seek relief at the hospital at such an advanced stage of the disease that a biopsy is considered by the attending physician as unnecessary for diagnosis. It is therefore believed that the incidence of labial carcinoma in Bihar is much higher than the 12.6 per cent suggested by an analysis of the biopsy material. An inquiry revealed that the inhabitants of Bihar, particularly men, are addicted to the use of *Khaini*, a mixture of a powder of dried tobacco leaf with lime. It is a usual sight to see men squatting or resting singly or in groups in the course of their work and rubbing carefully for several minutes a powder of dried tobacco leaf with a small amount of lime in the palm of the left hand, using the thumb of the right hand, until the desired mixture is obtained. Then they deposit a pinch of the mixture in the groove between the front teeth and the lower lip. It is left there until it gradually gets diluted with saliva and is swallowed. It is replaced by a fresh mixture at frequent intervals during the day. The changes which result in the mucosa have so far not been carefully studied but in a certain number of persons a superficial ulcerative process commences in a red, congested mucosa lining the gums and the lip. The cancer which supervenes is squamous cell carcinoma of the type usually seen in the buccal mucosa.

COMMENT

The three types of carcinoma described have not been commented on in the literature on cancer, except for a reference to the chutta carcinoma, and appear to be peculiar to the people of India. From what has been stated they may be assumed to be associated with the conditions prevalent in the country. Two similar types, in addition to these, have been described by previous observers. One, the Kangri cancer, caused by the carrying of an earthen pot containing burning embers close to the abdomen, observed among the inhabitants of Kashmir, has been familiarized to the medical and lay world by the many writings of Neve.[8] This custom owes its origin to the extreme poverty of the many ill clad Asiatic peoples, who have no other equally cheap means of keeping themselves warm during the rigors of a cold winter. A similar method of warming themselves obtains, for instance, among the Chinese (*Chang*) and the Japanese (*Kairo*). The second, the carcinoma of the scalp among the Mohammedans in Northern India, has been referred to by Sutherland[9] and was attributed by him to the irritation of the blunt razors used in shaving their heads. A cancer on the hairy part of the head is infrequent; according to European observations,[10] the cases represent only 0.8 per cent of all cases of cancer of the scalp and the face. It appears that in the Mohammedans of North Africa[11] who shave their heads carcinoma of the scalp is more common than in the other inhabitants of the same region.

The three types of carcinoma, namely, dhoti, chutta and khaini, immediately suggest to a superficial observer a relationship between chronic irritation and cancer and may appear to him as being merely additional examples illustrative of that relationship. This would be an unfortunate deduction, because, though it "pleases and satisfies the mind," it "does not make for progress."[12] The role of irritation in the production of cancer has been excellently reviewed recently by Berenblum[13] and by Oberling,[12] so that it is unnecessary to describe the clinical and experimental observations which have led to a clearer understanding of the part which irritation plays in the cancerous transformation of tissue cells. Certain facts bearing on this may, however, be set out here. If by "irritation" is meant the harmful influence of a physical agent or a chemical substance acting on living cells, then it is justifiable to suppose that irritation is not wholly without effect on the development of cancer. At the same time, it has never been satisfactorily demonstrated that irritation per se has ever been known to cause cancer. The experiments of Burrows[14] show that a nonspecific "chronic irritation" is not a factor in the causation of neoplasia. The nature of the irritant, therefore, plays a dominant part in the development of a cancerous condition. This has been referred to by Nicholson as the "factor of realisation" or stimulus.[15] The development of cancer,

8. Neve, E. F.: (*a*) Indian M. Gaz. **35**:161, 1900; (*b*) **59**:341, 1924.

9. Sutherland, D. W.: Arch. Middlesex Hosp. **3**:84, 1904.

10. Beck, S. C., in Jadassohn, J.: Handbuch der Haut und Geschlechtskrankheiten, Berlin, Julius Springer, 1933, vol. 12, pt. 3.

11. Roussy, G.; Nouveau traite de médecine, Paris, Masson & Cie, 1929, vol. 2. Brault, G.: Bull. Soc. path. exot. **6**:133, 1913.

12. Oberling, C.: The Riddle of Cancer, translated by W. H. Woglom, New Haven, Conn., Yale University Press, 1944.

13. Berenblum, I.: Arch. Path. **38**:233, 1944.

14. Burrows, H.: J. Path. & Bact. **43**:427, 1936.

15. Nicholson, G. W.: Lancet **1**:368, 1932.

however, is dependent not only on the influence of the so-called cancer-producing substances but also on the susceptibility of the tissues to the stimulus. To borrow the nomenclature of the experimental morphologists, the tissues must be "competent" to react to the point of a development of cancer, or must become so as a result of a series of changes which they have undergone after being subjected to influences which are not carcinogenic by themselves. This factor, which is overlooked in most discussions of the subject, has been referred to as the "factor of determination" or the reaction by Nicholson.[15] It is probable that most cells of the body would be competent to react by progressive growth and neoplastic change if a suitable substance "unmasked or activated the natural evocator already present in the competent tissue."[16] In other words, neoplasia is determined in the tissue in which the process of cancer is induced. There is one further conception which needs clearer comprehension and it is that the cells of any tissue may inherently possess or acquire the competence to react by tumor development and yet the requisite conditions may be wanting for the growth of tumor tissue. Conditions may exist which render cells neoplastic, but others may be lacking which lead to the proliferative and subsequent infiltrative behavior of the altered cells. This conception of a sharp distinction between tumor inception and tumor formation has resulted from the experimental work of Deelman and van Erp[17] and that of Mackenzie and Rous.[18] Unfortunately, the biologic character of competence is not recognizable by any cytologic characteristics at present known, and it is impossible to prejudge which cell or tissue will react with development of a tumor when subjected to a given stimulus. The recent studies of Craig and his co-workers[19] suggest that "a characteristic enzymic change in tissue cells may herald malignant or premalignant transformation for a considerable time (weeks or months) before malignant changes can be recognized by microscopic examination." During the last thirty years it has been possible experimentally to induce tumors in susceptible laboratory animals by exhibition of most varied chemical and physical carcinogenic agents. The induced tumors do not show differences in structure corresponding to the difference in character of the carcinogens. The work of experimental embryologists has shown that "evocators" or substances that induce development can be extracted from the tissues of the embryo. The specificity of the type or the form of the tissue induced does not depend on the evocating substance or the stimulus but is determined by the reacting tissues of the developing embryo. The embryonal tissue must be in just the right state—neither too young nor too old—to react at all; otherwise, the most constant stimulus is impotent to induce its physiologic effect.[20] Similarly identical tumors may be induced with the most varied agents, such as heat, light, roentgen rays, gamma rays and chemical substances; nor would it be possible to distinguish them from tumors occurring spontaneously at the same sites. It has therefore been assumed that carcinogenic substances, whether introduced from outside the organism or elaborated inside it, gradually but irrevocably alter the metabolism of cells which will become cancerous, by producing suitable changes in their milieu.

Much experimental work has shown that prolonged application of a specific irritant (carcinogen) to the skin of laboratory mice leads to increased vascularity of the area, particularly in the proximity of the basal layer of the epidermis, followed by marked widening of the blood capillaries. The blood flow in these dilated channels in the dermis is slowed and later, as a result of the formation of capillary thrombi, as well as of the pressure exerted by the extravascular tissue, rendered very sluggish. In certain regions not only is the circulation slowed but the fluid circulating through the capillaries becomes different in character. The fluid slowly seeping through the ectatic blood capillaries contains hardly any particulate material, being almost entirely composed of fluid plasma. This condition has been described as "plasma skimming." It has been suggested by Kreyberg[4] that the initial hyperemia when continued for a certain length of time leads to nonspecific changes in the skin, such as thickening of the epidermis, absorption of the subcutaneous fat and atrophy and thinning of the skin, followed by hyperplasia of the hair follicles and development of papillary proliferations on the skin. The subsequent ectasia of vessels, capillary thrombosis and associated connective tissue fibrosis lead to interference with the normal respiratory processes of the overlying epidermal cells. If the successive generations of these cells are unable to adapt themselves to the altered metabolic

16. Nicholson, G. W.: Guy's Hosp. Rep. **19**:271, 1939.
17. Deelman, H. T., and van Erp, J. T.: Ztschr. f. Krebsforsch. **24**:86, 1926.
18. Mackenzie, I., and Rous, P.: J. Exper. Med. **73**: 411, 1941.
19. Craig, F. N.; Bassett, A. M., and Salter, W. T.: Cancer Research **1**:869, 1941.
20. Needham, J.: Biochemistry and Morphogenesis, London, Cambridge University Press, 1942.

conditions, necrosis and ulceration of the surface skin supervene; if, however, some of the epidermal cells adopt an alternative metabolism for obtaining the necessary energy for growth and continuation and are able to transmit this character to their progeny, neoplasia or tumor formation results. These views regarding the altered metabolic activities of neoplastic cells are supported on the one hand by the investigations of Warburg [21] and his co-workers on the glycolytic metabolism of tumor cells and on the other hand by observations on the cultivation of tumor cells in vitro. It appears that anaerobic and even aerobic glycolysis is a characteristic property of many cancer cells. It has also been shown by Fischer [22] that whereas normal cells (fibroblasts and epithelial cells) are unable to survive for long in homologous plasma alone, cancer cells have the property of elaborating all the substances necessary for their growth from a heterologous serum and are therefore able not only to survive but to proliferate in such a nutrient medium without the addition of any other constituent.

If the considerations outlined in the foregoing paragraphs are applied to the three types of cancer described at the commencement of this paper, certain interesting facts emerge which may be discussed at this stage.

Dhoti Cancer.—It is difficult to speculate on the nature of the substance or substances which are responsible for initiating the changes which result in the inception and the growth of this type of cancer.

> . . . There is no evidence that tumors are unduly prevalent at sites of uncomplicated manifestations of chronic mechanical irritation (e. g., corns or calluses), nor is it possible to induce tumors in animals by friction alone. Chronic mechanical irritation (friction) cannot, therefore, be accepted as a common or even a likely cause of tumor formation.[13]

It must, however, be pointed out that at the sites of dhoti cancer described the mechanical friction or the pressure is associated with an accumulation of desquamated epithelium soaked in abundant sweat. This material remains in contact with the skin, which is subjected to continual mechanical stresses. Moreover, the inadequate cleansing of the body, owing to the customs of the people, does not assist the removal from the affected area of substances which are not easily soluble in water.

It has been stated in the foregoing pages that the skin at the sites of pressure of the dhoti shows evidence of hyperplasia and hyperkeratinization of the epithelium. The fat on the epidermis is rich in cholesterol and its esters, which amount in these structures to 16 per cent of the lipoids.[23] Several tinctorial and chemical studies of the skin [24] have demonstrated the presence of highly unsaturated hydrocarbons in the epidermis. In a recent study Burtenshaw [25] stated that:

> . . . fat from the palmar skin and nails, where there are no sebaceous glands, contains oleic acid and other unsaturated acids with their esters and soaps, and traces of the short chain fatty acids; whereas fat from skin supplied with sebaceous glands, from hair and from cerumen contains numerous long and short chain fatty acids with their esters and soaps derived from, or characteristic of, sebum.

Twort and Bottomley [26] have shown that oleic acid, an unsaturated fatty acid with a double bond in the middle of a straight carbon chain and a terminal carboxyl group, is an effective stimulus to sebaceous cells in laboratory mice, leading to hyperplasia of surface epithelium and even sebaceous carcinoma. It was also shown by them that oleic acid seemed to "materially increase the carcinogenic potency of chrysene and other weak agents." Oleic acid can be cyclized to civetone, which could then give a sterol ring system through hexahydrocivetene and dimethylhexahydrocivetone.[20] It has also been shown by Needham [27] that "implantation of triglycerides mixed with small amounts of sterols such as cholesterol gives rise to swellings of a characteristic cuboidal epithelium" in the growing amphibian embryo, simulating the action of the evocators. This may suggest, although it does not prove, that from desquamated epithelium bathed in stale sweat substances may be formed which in susceptible persons supply the necessary stimulus to the inception of neoplasia in a skin suitably altered for the purpose.

The histologic appearance of specimens of skin from the loins of some persons wearing the dhoti shows a remarkable similarity to the changes produced in the dermis of mice after repeated applications of carcinogens; scratchings and mild abrasions may then initiate the conditions necessary for tumor growth in persons whose skins

21. Warburg, O.: The Metabolism of Tumors, translated by F. Dickens, London, Constable & Co., Ltd., 1930.
22. Fischer, A.: Ztschr. f. Krebsforsch. **26**:228, 1928.
23. Unna, P. G., and Golodetz, L.: Biochem. Ztschr. **20**:469, 1909.
24. Partridge, R. A.: J. Am. Leath. Chem. A. **33**:144, 1938. Koga, K.: Fukuoka Acta med. **27**:124, 1934; cited by Burtenshaw.[25]
25. Burtenshaw, J. M. L.: J. Hyg. **42**:184, 1942.
26. Twort, C. C., and Bottomley, A. C.: Lancet **2**: 776, 1932.
27. Needham, J.; Waddington, C. H., and Needham, D. H.: Philos. Tr. Roy. Soc., London, s.B **114**:393, 1934.

have acquired competence for cancerous proliferation of epithelial cells.

It may not be out of place here to suggest that by analogy the action of carcinogenic substances as observed in experimental animals is being uncritically accepted as the only determinant of the spontaneous development of tumors in man. The brilliant studies of Cook, Kennaway and others in the isolation and the synthesis of carcinogenic chemicals has distracted attention from the factor of determination in the induction of tumors. This was not altogether overlooked even by earlier observers. Nearly eighty years ago Curling [28] stated that "the soot does not appear to generate epithelial cancer but by its continued contact to produce a state of skin favourable to the development of this disease." Leitch [29] was struck with the fact that in chimney sweeps, other parts of the body were as much, if not probably more, exposed to the action of the soot as the scrotum, and though soot warts were occasionally seen on the skin at other places, soot cancer in the chimney sweeps was mainly confined to the scrotum. It is further interesting to note that in the occupational cancer of the Scottish shale oil industry Scott [30] found that:

> . . . epitheliomata which occur in oil workers and labourers (who do not come into such intimate contact with paraffin in the course of their work) as distinguished from paraffin workers, without any primary condition of dermatitis are, as a rule, found among those men, such as retortmen, labourers, stillmen, oil workers, etc., who come into contact with ash, coke or other gritty material. The scrotum is most frequently the site of such lesions, this being due to the difficulty of ensuring cleanliness of this region.

It can therefore be stated that exposure to a carcinogenic substance is only one factor in the induction of cancer and that the competence of the tissues to react with neoplasia is a factor of equal, if not of greater, importance.

Chutta Cancer.—The available evidence that thermal irritation can lead to the production of tumors, is, for many reasons, more convincing than that implicating mechanical injuries. It is unlikely that the neoplastic condition in chutta cancer starts at the site of a former burn and the resulting scar. The way in which the chutta is held in the mouth probably does not allow accidental contact of the glowing end with the mucous membranes of the palate. With a view to finding out the heat to which the mucous membrane is exposed during the process of smoking the temperature records given in table 4 were taken in 4 cases with the bulb of a long stem thermometer held close to the lighted end of the chutta in the mouth. As may be seen, the temperature was quite bearable, and in the vicinity of 65 C. (149 F.) only. It is interesting to note that the temperature to which the skin was found to be exposed with a kangri was noted by Neve [8a] to be between 66 and 93 C. (150 and 200 F.). It is unlikely that such temperatures would lead to burning or charring of the skin. In the case of the chutta cancer, apart from the thermal factor, the exposure of the mucous membranes to the substances elaborated by the burning of the tobacco leaf has also to be taken into consideration. Similarly, in the case of kangri cancer it should be noted that the shirts of most Kashmiris who use the kangris are impregnated with sweat, smoke and soot, and they have few opportunities of changing their clothes or bathing themselves during the winter months. Neve [8] stated that "elderly Kashmiris exhibit small localised papules or macules. These are dry, slightly scaly and usually pigmented" lesions not unlike those seen in the loins of people wearing the dhoti.

It may therefore be suggested that the heat from the kangri or the chutta has only a cocarcinogenic action, augmenting the effect of some carcinogenic substance or substances. And the usual impression of chutta and kangri cancer arising from burns appears, therefore, to be erroneous. A certain amount of support for this conclusion is lent by the findings of Parodi [31] to the effect that if the skin of experimental animals had previously been heated to 70 C.

TABLE 4.—*Temperature in the Space Below the Palate Near the Burning End of a Chutta*

Patient	Age, Yr.	Temperature Readings: 1st		2d		3d		Mean	
Hindu female of Guramma	14	63.0 C.	145.4 F.	62.2 C.	143.9 F.	64.0 C.	147.2 F.	63.1 C.	145.5 F.
Hindu female of Somulamma	20	72.4 C.	162.3 F.	68.0 C	154.4 F.	62.0 C.	143.6 F.	67.5 C.	153.5 F.
Hindu female of Nagamma	22	62.0 C.	143.6 F.	65.0 C.	149.0 F.	70.0 C.	158.0 F.	65.7 C.	152.2 F.
Hindu female of Mariamma	35	70.0 C.	158.0 F.	62.0 C.	143.6 F.	65.0 C.	149.0 F.	65.7 C.	152.2 F.

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(158 F.) for several months, the production of tar tumors was delayed, whereas heating the epidermis to 50 C. (122 F.) favored the development of cancer.

Khaini.—We have unfortunately had no opportunity to study the early lesions of khaini cancer either clinically or histologically. Any discussion of the subject at this stage would not yield useful information.

SUMMARY

Three new types of cancer are found in different regions of India, namely, Bombay, Vizagapatam and Patna. They may be called the dhoti, chutta and khaini cancers. They are associated with the wearing of a light garment (the dhoti), the smoking of a cigar (the chutta) with the lighted end in the mouth and the depositing of tobacco and lime (khaini) behind the lower lip of the mouth. A histologic study of the lesions which precede the development of two of these cancers reveals a similarity of appearance between them and the precancerous stages in mice which have been painted experimentally with carcinogenic substances.

The observations reported and a review of the available experimental literature on the subject of changes in the skin as a result of exposure to mechanical and thermal irritants lead to the conclusion that in the induction of the tumors described the part played by the reaction of the tissues which are the seat of the cancer is equal in importance to that played by the carcinogenic substances themselves.

EXPERIMENTAL NEPHROPATHIES

V. A COMPARISON OF THE TOXIC ACTION OF URANIUM, MERCURY AND CHROMIUM ON THE KIDNEYS

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In a previous paper [1] in this series attention was called to the differences in the gram atom concentrations of uranium, mercury and chromium, respectively, in the salts uranyl nitrate ($UrO_2[NO_3]_2$), mercury bichloride ($HgCl_2$) and potassium dichromate ($K_2Cr_2O_7$). In these three chemical agents, which were used in our experiments, it was presumably uranium, mercury and chromium in their respective molecules that produced nephrotoxic effects in the dogs poisoned with them. It was suggested that "perhaps a more accurate comparison of the toxic effects of these poisons could be made if the amount of each substance injected was proportional to the gram atom equivalent of the respective metal in the molecule than if merely an equal number of milligrams was used." This system of units is particularly convenient for comparison when definite atomic proportions of elements are desired, because gram atoms of all elements contain the same number of atoms.

It is possible that this principle may also be applied to a comparison of the degrees of toxicity of any group of poisons when it is known that one ion of the substance is responsible for the toxic effect. In the cyanides, for example, the cyanogen (CN) ion is the toxic portion of the molecule. One milligram of sodium cyanide (NaCN) contains 10.80×10^{-3} gram ions of cyanogen, while 1 mg. of potassium cyanide (KCN) contains 8.16×10^{-3} gram ions of cyanogen. Theoretically, sodium cyanide should be more poisonous because "the toxic effects of these alkali cyanides are the same as those of the equivalent amount of HCN which they contain." [2] Sodium and potassium chlorate are poisonous and presumably it is the chlorate ion in the molecule that is toxic. One milligram of sodium chlorate ($NaClO_3$) contains 7.36×10^{-3} gram ions of ClO_3 while a similar amount of potassium chlorate ($KClO_3$) contains only 5.57×10^{-3} gram ions of ClO_3. This difference may account for the fact that the sodium salt is more prone to produce methemoglobin in vivo than is potassium chlorate.[3]

In our experiments we did not use doses of the poisons in the proportion of the gram atom equivalents of the metals in the respective molecules. However, after transforming the doses employed into their gram atom equivalents we found that we did have some evidence regarding the relative toxicity of uranium, mercury and chromium. This has enabled us to evaluate differences in the toxic effects produced by the three substances used in our experiments. The method of calculation appears to be worthy of reporting.

The methods used in these experiments have been described in a previous paper.[1] Each poison was dissolved in isotonic solution of sodium chloride and administered intravenously. The strength of the solution was such that each animal received a known quantity of the poison, ranging from 0.01 to 3.0 mg. per hundred cubic centimeters of blood. The actual concentration of the poison in the plasma was calculated on the basis that the plasma constitutes 60 per cent of the total blood volume.[4] One milligram per hundred cubic centimeters of blood would yield a concentration of 16.67×10^{-3} mg. of the poison per cubic centimeter of plasma. The poison would appear in the same concentration in the glomerular filtrate, since the glomerular walls are freely permeable to crystalloids. It is more difficut to calculate the concentration of the poison as the filtrate passes through the proximal convoluted tubules, since available evidence does not support the idea that the tubules tend to reabsorb a constant fraction of the filtrate, but rather it appears that they tend to return water to the peritubular blood until that blood has some specific composition or until the urine has

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reached some specific concentration.[5] For purposes of calculation, however, one may accept the statement of Richards[6] that the proximal convoluted tubules absorb about 20 per cent of the water from the filtrate. On this basis, the concentration of the poison in the lumens of the distal ends of the proximal convoluted tubules would be about 20.84×10^{-3} mg. per cubic centimeter.

One milligram of uranyl nitrate ($UrO_2[NO_3]_2$) contains 2.53×10^{-3} gram atoms of uranium;[7] 1 mg. of mercury bichloride ($HgCl_2$) contains 3.66×10^{-4} gram atoms of mercury, and 1 mg. of potassium dichromate ($K_2Cr_2O_7$) contains 6.80×10^{-3} gram atoms of chromium. From these values it is possible to calculate the approximate gram atom concentration of each of the metals in the plasma, in the filtrate and in the urine at the distal ends of the proximal convoluted tubules. These values are shown in the accompanying table.

On the basis of the data in the table alone it appears that the atom of uranium is about 50 per cent more toxic than that of mercury and the atom of mercury about twice as toxic as that of chronium. One milligram of uranyl nitrate with only 0.42×10^{-4} gram atoms of uranium per cubic centimeter of plasma and filtrate caused approximately as much necrosis as 2 mg. each of mercury bichloride and potassium dichromate with 1.22×10^{-4} and 2.24×10^{-4} gram atoms of mercury and chromium, respectively, per cubic centimeter of plasma and filtrate. Four plus necrosis was produced by 2 mg. of uranyl nitrate with 0.84×10^{-4} gram atoms of uranium per cubic centimeter of plasma, while 3 mg. of mercury bichloride with 1.83×10^{-4} gram atoms of mercury, and 4 mg. of potassium dichromate with 4.48×10^{-4} gram atoms of chromium, respectively, were required to produce such a marked degree of necrosis. The metals in the inorganic chemical agents used in our experiments can be arranged in the order of increasing toxic effects on the kidneys as follows: chromium, mercury and uranium. It may or may not be significant, but this is also the order of their atomic numbers: chromium, 24; mercury, 50; uranium, 92.

This simple method of studying the relative toxicity of various substances does not, however, take into consideration certain other factors that may influence the action of a poison. Chemical agents differ in their ability and tendency to combine with proteins and other constituents of the plasma. When the quantity of combined poison in the plasma is high, the amount of free and, therefore, filtrable poison will be reduced. This may alter markedly the force of the insult to the kidneys because of the lower concentration of the chemical agent in the filtrate. The location of the metal in the molecule may also affect its toxicity. Of the chemical agents used in our experiments, uranium and mercury were cations while chromium was in the anion of its molecule. There may be differences in the degree of diffusibility among poisons that are not taken into account in this method of comparing toxicity.

A Comparison of the Toxic Action of Uranium, Mercury and Chromium on the Kidneys Measured by the Extent of Necrosis

Doses of Chemical Agent	Degree of Necrosis	Gram Atoms per 1 Cc. of Plasma and Filtrate	Gram Atoms per 1 Cc. of Urine at Distal Ends of Proximal Convoluted Tubules
$UrO_2(NO_3)_2$			
1 × 1 mg.	2 and 3 plus	0.42×10^{-4}	0.52×10^{-4}
1 × 2 mg.	3 and 4 plus	0.84×10^{-4}	1.04×10^{-4}
1 × 3 mg.	4 plus	1.26×10^{-4}	1.56×10^{-4}
2 × 2 mg.	.....	1.68×10^{-4}	2.08×10^{-4}
2 × 3 mg.	4 plus	2.52×10^{-4}	3.12×10^{-4}
$HgCl_2$			
1 × 1 mg.	1 and 2 plus	0.61×10^{-4}	0.76×10^{-4}
1 × 2 mg.	2 and 3 plus	1.22×10^{-4}	1.52×10^{-4}
1 × 3 mg.	4 plus	1.83×10^{-4}	2.28×10^{-4}
2 × 2 mg.	4 plus	2.44×10^{-4}	3.04×10^{-4}
2 × 3 mg.	.....	3.66×10^{-4}	4.56×10^{-4}
$K_2Cr_2O_7$			
1 × 1 mg.	1 plus	1.12×10^{-4}	1.42×10^{-4}
1 × 2 mg.	2 and 3 plus	2.24×10^{-4}	2.84×10^{-4}
1 × 3 mg.	2 and 3 plus	3.36×10^{-4}	4.26×10^{-4}
2 × 2 mg.	4 plus	4.48×10^{-4}	5.68×10^{-4}
2 × 3 mg.	4 plus	6.72×10^{-4}	8.52×10^{-4}

SUMMARY

The gram atom or the gram ion concentration of the known toxic portion of a molecule of poison furnishes a simple method of comparing the relative toxicity of chemical agents when there is a relatively accurate means of comparing their minimum and maximum effects.

5. Smith,[4] p. 202.

6. Richards, A. N.: Methods and Results of Direct Investigations of the Functions of the Kidney, Baltimore, William & Wilkins Company, 1929; Urine Formation in the Amphibian Kidney, in Harvey Lectures, 1934-1935; ibid., 1935, vol. 30, p. 93.

7. When the water of crystallization in the molecule is taken into account, 1 mg. of $UrO_2(NO_3)_2.6H_2O$ contains 1.99×10^{-3} gram atoms of uranium. The concentration in the plasma and in the filtrate and in the urine at the lower ends of the proximal convoluted tubules would be 0.33×10^{-4} and 0.41×10^{-4} gram atoms of uranium, respectively, per cubic centimeter.

THE BONE MARROW IN "FOLIC ACID" DEFICIENCY AND ITS RESPONSE TO CRYSTALLINE LACTOBACILLUS CASEI FACTOR ("FOLIC ACID")

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Granulocytopenia has been reported as occurring in rats given certain purified diets,[1] and it has been shown to be corrected by crystalline Lactobacillus casei factor ("folic acid"). The incidence of granulocytopenia is greatly increased by the inclusion of certain sulfonamide compounds in the purified diet,[2] and under these conditions anemia also occurs. These dyscrasias have responded to treatment with liver concentrates and with crystalline "folic acid."[3] The lesions seen in these rats have been reported,[4] including progressive hypoplasia of bone marrow, with aplasia in the cases in which the effects are extreme. No detailed studies of the hemopoietic system have been published.

The present studies were undertaken for the purpose of examining the anatomic basis of these experimental dietary blood dyscrasias. As a preliminary study, the normal myelogram of the rats used in this laboratory was determined.[5] A method of estimating the proportion of active marrow was devised and was used to correct the differential marrow count so as to quantitate the various components of the bone marrow. The present study was divided into three phases. The first was the study of the marrow of leukopenic rats and its response to "folic acid." The second was the study of the marrow during the development of the dyscrasias. The third was the study of the marrow in rats showing both leukopenia and anemia and the response of the marrow to treatment with "folic acid."

From the Pathology Laboratory (K. M. Endicott and Maurine Ott) and the Division of Physiology (Floyd S. Daft), National Institute of Health.

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EXPERIMENTAL METHODS

In this laboratory a large number of rats are maintained on a certain regimen to provide leukopenic and anemic animals for the purpose of testing various therapeutic agents. At weaning, rats of Wistar, Osborne and Mendel, and New Haven strains are fed a purified diet consisting of purified casein (18 per cent), dextrose (72 per cent), hydrogenated vegetable oil (3 per cent), cod liver oil (2 per cent), salt mixture (4 per cent), and succinylsulfathiazole (1 per cent), with a daily vitamin supplement of thiamine hydrochloride (100 micrograms), pyridoxine hydrochloride (100 micrograms), riboflavin (200 micrograms), calcium pantothenate (200 micrograms), nicotinic acid (1 mg.), choline chloride (10 mg.), vitamin K (20 micrograms) and biotin (0.5 microgram). In rats restricted to this regimen, leukopenia and granulocytopenia regularly develop and anemia less regularly. Leukocyte counts are made periodically, on the basis of which rats are selected for testing purposes.

In order to study the marrow of rats with full blown granulocytopenia, 13 such rats were killed when their polymorphonuclear leukocyte counts fell below 150 per cubic millimeter. A corresponding group of 19 rats with similar or more severe granulocytopenia were given daily oral doses of 25 micrograms of crystalline "folic acid" for periods of two, three, or four days. At the expiration of each period of treatment blood counts were made and a subgroup of the rats were killed and examined.

A second series of 13 rats with both granulocytopenia and anemia were killed and examined for comparison with rats showing granulocytopenia alone. Thirteen other rats with similar or more severe granulocytopenia and anemia were treated with daily oral doses of 25 micrograms of crystalline "folic acid" for two, four, eight or twelve days. At the end of each period

studies of the tail blood were made, and a subgroup of the rats were killed and examined.

For the purpose of studying the development of the dyscrasias, the following regimen was adopted after preliminary experiments showed that weanling rats were unsatisfactory. (Weanling rats present a myelogram quite different from that of rats aged 2 months or more. It was found too difficult to separate some of the observed changes into those due to the diet and those occurring as a result of the normal change from infantile to adult marrow.) Seven litters of rats were given a stock diet for twenty-four to thirty-six days to allow a change from infantile to adult myelogram. They were then fed the succinylsulfathiazole-containing diet. Three litters were killed after thirty-eight, thirty-nine and forty-three days, respectively, and three more litters were killed after forty-six days of the experimental diet. A seventh litter was followed carefully with periodic blood counts, and individual members of the litter were killed at various intervals in order to demonstrate that the various stages of the dyscrasia could be produced in the same litter.

The last blood count of each rat was made from tail blood taken on the day of death. Leukocyte and erythrocyte counts were made in duplicate. Differential counts were made by the meander method on smears stained with Giemsa stain. Hematocrit determinations were made with the Van Allen hematocrit, 1.3 per cent sodium oxalate being used. Reticulocyte counts were made with the Miller counting disk. Rats were killed by decapitation or exposure to illuminating gas. Tissues were fixed in Orth's fluid. Heart, lungs, liver, spleen, pancreas, adrenal glands, kidneys and quadriceps muscle were embedded in paraffin, sectioned and stained with hemalum-azure-eosinate and with iron hematoxylin–picrofuchsin. The right femur and the thoracic part of the spinal column were decalcified in 5 per cent formic acid, embedded in pyroxylin or paraffin, sectioned, stained with toluidine blue–eosinate [6] or with hemalum-azure-eosinate and iron hematoxylin–picrofuchsin. Giemsa-stained smears of marrow from the left femur were prepared by the plasma diluting method.[7] From 500 to 1,000 cells were classified. In the studies on the development of the lesions the following additional material was studied: cervical lymph nodes, thymus and fat stains of frozen sections of the thoracic part of the spinal column. The submaxillary and posterior cervical lymph nodes and the thymus were dissected clean, weighed in the wet state, and fixed and studied histologically.

The sections of the femur and the spinal column were studied to determine the proportion of active marrow by a method described in detail in a previous report.[5] This consists of sketching the areas occupied by active marrow, fat, vasa, bone trabeculae and total marrow space with the aid of a projection apparatus. Transverse bands of the midshaft, the distal part of the shaft and the distal epiphysis of the femur and of the dorsal vertebral marrow cavity were sketched, and the areas occupied by each of the components were determined by the use of finely ruled graph paper. The proportion of active marrow was determined for each band by the formula:

$$\text{Proportion of active marrow} = \frac{\text{area occupied by active marrow}}{\text{(total area of strip)} - \text{(area occupied by bone)}}$$

The mean value of these four calculations is given in the tables. This mean value was used to calculate the "index of the total quantity" of the cells of each type in the marrow differential by the formula:

Index = mean proportion of active marrow × percentage in marrow smear

This index is a semiquantitative measurement of the total quantity of the cells of each type in the marrow and serves to correct the marrow differential in a fashion similar to the correction of the differential blood count achieved by making a total leukocyte count. Thus, if the mean proportion of active marrow is 0.10 and the percentage of erythroid cells in the marrow smear is 75.0, the index will be 7.5. Comparison of this with the normal index of 30.8 indicates an absolute decrease of erythroid cells despite a relative increase in the differential count.

Megakaryocytes were studied by inspection of marrow sections and were not determined quantitatively.

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RESULTS

In the tables the results of the various studies are presented as mean values with standard deviations to conserve space. Standard deviations were calculated from the formula: S.D. $= \sqrt{\frac{S\,(x^2)}{n-1}}$. Findings in individual animals are not shown except for one litter but are mentioned occasionally. Each phase of the study will be dealt with separately, and an attempt will be made to correlate them.

Marrow in Granulocytopenia.—The peripheral blood in the 13 rats with granulocytopenia showed an average leukocyte count of 1,540 per cubic millimeter at the time of death, with 50 neutrophilic leukocytes, 1,490 lymphocytes and 30 nucleated red cells. The mean hematocrit value was 41.1 (table 1).

The marrow was hypocellular. The mean proportion of active marrow was 0.352, compared with a normal of 0.827. The marrow cavity was filled largely by dilated blood channels and by edema fluid. Fat was conspicuously absent. Neutrophilic granulocytes of all types were markedly decreased. Eosinophilic granulocytes were also decreased. The index of total myeloid cells was 4.6, compared with a normal of 52.0 Erythroid cells were decreased to 17.1, compared with a normal index of 30.8 The myeloid-erythroid ratio was reversed, being 0.45, compared with a normal of 1.93. Lymphocytes, blasts and apparently megakaryocytes were decreased. There was a slight to marked increase in reticuloendothelial cells and in plasma cells. As can be noted by the standard deviations, there is considerable variation in the values obtained. This is due largely to the occurrence of relatively slight hypoplasia in 2 rats and of extreme hypoplasia in several rats. No rats showed hyperplasia, and none showed any increase of granulocytes of any form.

In addition to the changes noted in the blood and the marrow, there was noted regularly lymphoid exhaustion of the spleen and absence

of splenic hemopoiesis. Necrosis and calcification of skeletal muscle occurred regularly. Pulmonary infections were present in 3 rats. Only 1 rat showed necrosis of the liver, but 5 showed hydropic degeneration of liver cells. These lesions have been reported elsewhere.[4a]

Effect of "Folic Acid" on the Marrow of Granulocytopenic Rats.—The response of the hemopoietic system to "folic acid" was rapid and spectacular. The total leukocyte count began to rise on the first or second day of treatment. The average count before treatment was 1,600 per cubic millimeter. The average counts on the second, third and fourth days of treatment were 4,070, 4,310 and 11,110, respectively. The neutrophils showed an increase from 10 per cubic millimeter before treatment to 1,060, 1,370 and 4,550 on the second, third and fourth days. Eosinophils, lymphocytes and nucleated red cells increased. The hematocrit value decreased somewhat.

The marrow of these treated rats as compared with that of the 13 untreated rats became hypercellular and showed a prompt return of myeloid elements. The ratio of myeloid to erythroid elements was approximately normal after two to three days but reached as high as 30:1 in 1 rat in four days. This marked myeloid hyperplasia may have produced the slight decrease in hematocrit value by crowding out erythroid elements temporarily. In the myeloid series, all forms increased, especially premyelocytes and myelocytes, which reached values two to three times larger than normal. Blasts increased slightly but there was no evidence that they contributed to any significant degree in the regeneration of the myeloid series. Lymphocytes, reticuloendothelial cells and plasma cells decreased. Megakaryocytes appeared to increase.

The spleens of the treated rats showed lymphoid regeneration and marked splenic hemopoiesis. The other organs were similar to those of the untreated rats.

Three rats failed to respond to treatment with "folic acid." In these rats the peripheral blood showed progressive leukopenia and granulocytopenia and, in addition, severe anemia, which developed during the four days of treatment. The marrow was even more hypoplastic and depleted than that in the group of untreated rats. These rats showed bacteremia and pulmonary infections.

TABLE 1.—*Bone Marrow Indexes in Experimental Granulocytopenia Before and After "Folic Acid" Treatment (Means and Standard Deviations)*

	22 Normal Adult Rats	13 Rats Given No "Folic Acid"	1 Rat After 2 Days of "Folic Acid" Therapy	4 Rats After 3 Days of "Folic Acid" Therapy	11 Rats After 4 Days of "Folic Acid" Therapy	3 Rats After 4 Days of Unsuccessful "Folic Acid" Therapy
Peripheral Blood:						
Hematocrit reading	45.6 ± 3.9	41.1 ± 6.5	50.9	39.7 ± 6.1	34.0 ± 3.0	16.9 ± 2.2
White blood cells per cu. mm.	14,750 ± 5,110	1,540 ± 700	4,070	4,310 ± 1,930	11,110 ± 5,900	575 ± 10
Polymorphonuclears per cu. mm.	3,180 ± 1,970	50 ± 36	1,060	1,370 ± 1,040	4,530 ± 3,910	5 ± 5
Eosinophils per cu. mm.	240 ± 171	0 (2 rats had 20)	110	480 ± 530	270 ± 330	0
Lymphocytes per cu. mm.	10,840 ± 4,332	1,490 ± 700	2,900	2,400 ± 980	6,310 ± 2,880	570 ± 345
Normoblasts per cu. mm.	0 (1 rat had 40)	30 ± 6	130	1,140 ± 360	460 ± 670	30 ± 30
Proportion of active marrow	0.827 ± 0.021	0.352 ± 0.236	0.769	0.781 ± 0.156	0.902 ± 0.025	0.270 ± 0.060
Index of Total Quantity in Marrow: *						
Neutrophilic segmented forms and metamyelocytes (index)	34.5 ± 6.2	0.4 ± 0.8	4.8	5.9 ± 4.0	33.4 ± 14.3	0.1 ± 0
Neutrophilic myelocytes and premyelocytes (index)	9.2 ± 3.1	2.6 ± 2.3	16.0	13.4 ± 10.7	24.8 ± 11.4	0.1 ± 0
Eosinophilic granulocytes of all types (index)	8.3 ± 5.3	1.6 ± 3.3	15.4	7.8 ± 3.0	5.5 ± 4.3	0.2 ± 0.1
Total myeloid cells (index)	52.0 ± 24.8	4.6 ± 5.4	36.2	27.0 ± 11.6	60.9 ± 19.2	0.4 ± 0.1
Erythroid cells of all types (index)	30.8 ± 2.5	17.1 ± 15.6	30.3	37.7 ± 14.2	19.5 ± 17.8	19.6 ± 7.3
Myeloid-erythroid ratio	1.93 ± 0.93	0.45 ± 0.48	1.20	1.06 ± 0.59	8.40 ± 28.8	0.02 ± 0.02
Blast cells of all types (index)	1.9 ± 0.6	0.7 ± 1.1	2.5	2.4 ± 1.2	1.9 ± 0.8	1.1 ± 1.5
Lymphocytes of all types (index)	13.3 ± 4.3	8.5 ± 5.2	5.9	4.8 ± 3.4	6.1 ± 4.3	1.2 ± 0.7
Miscellaneous cells † (index)	2.0 ± 1.1	4.3 ± 4.8	1.9	6.0 ± 6.3	1.9 ± 1.3	4.9 ± 5.3

* See the text for the method of determining the index.
† Reticuloendothelial cells, plasma cells, mast cells, megakaryocytes and unidentified cells are included.

Marrow in Developing Granulocytopenia.—Changes in the blood and hemopoietic tissues began to appear in some rats after one month of the succinylsulfathiazole-containing diet. The time of onset varied even among the members of a single litter. The state of the marrow was closely correlated with that of the peripheral blood. For these reasons, the rats have been grouped according to the number of circulating neutrophils rather than by litters. In the group of rats with more than 1,000 neutrophils per cubic millimeter (mean, 1,690) there were definite changes in the marrow despite the fact that the peripheral blood was within the low normal range (table 2). These early changes in the marrow were in the myeloid series and consisted of a decrease of the total granulocytes.

particularly of the myelocytes and the premyelocytes. This might be considered a "right shift" on casual inspection, but in reality all forms were decreased.

depletion until practically none remained. Myelocytes and premyelocytes persisted longer than the more mature forms. The blasts and the lymphocytes decreased somewhat.

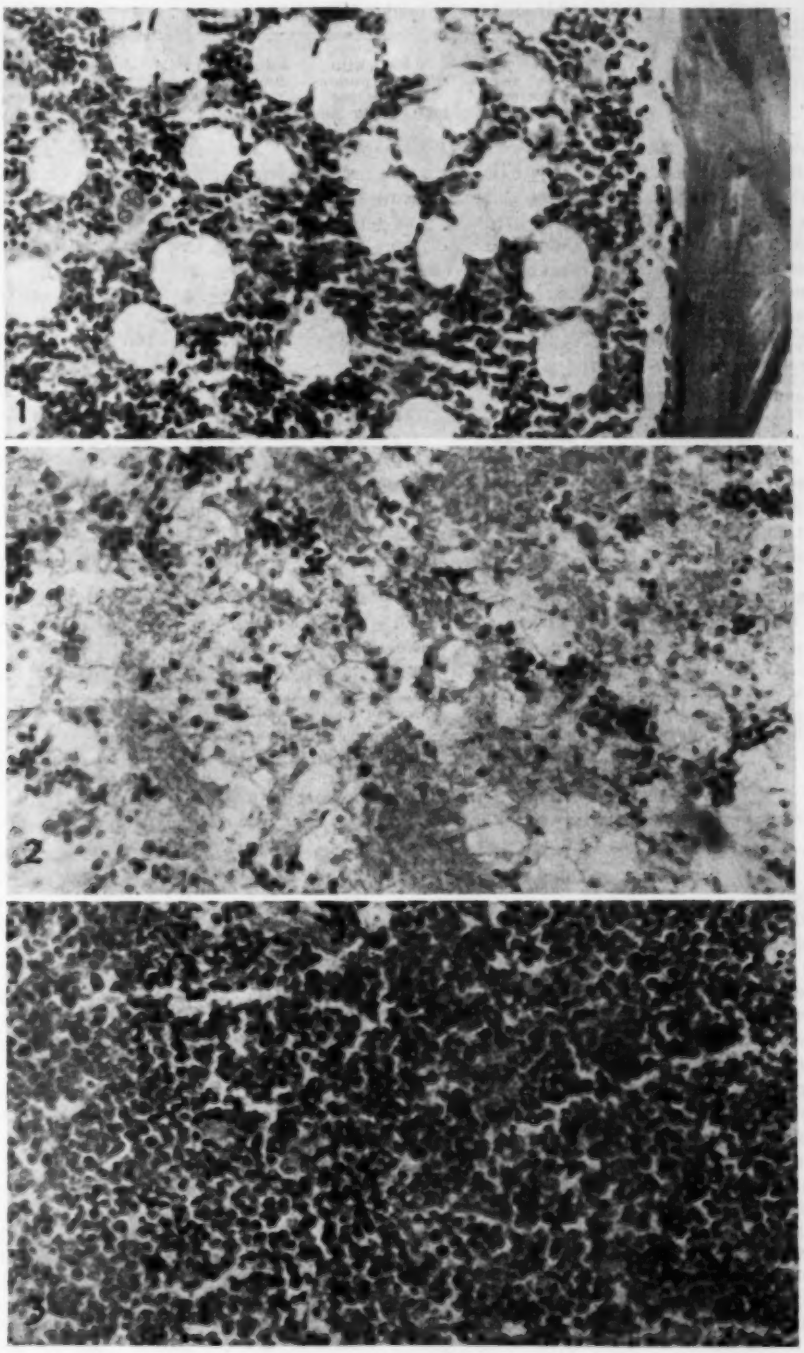

Fig. 1.—Photomicrograph of femoral marrow from a normal rat; Van Gieson stain; × 250.

Fig. 2.—Femoral marrow from a rat having agranulocytosis; Van Gieson stain; × 250. Note the marked hypocellularity and congestion.

Fig. 3.—Hyperplastic femoral marrow from a rat after four days' treatment with "folic acid"; Van Gieson stain; × 250.

As the circulating neutrophils decreased, the myeloid series in the marrow showed progressive

It appears significant that the erythroid series behaved quite differently in these older rats.

There was an absolute increase of erythroid elements in the marrow to as high as 73.0 in 1 rat (normal index, 30.8). The increase was largely from weaning. It is evident that the increase in erythroid elements prevented any striking decrease in total cellularity of the marrow.

TABLE 2.—*Bone Marrow Indexes During Development of Experimental Granulocytopenia (Means and Standard Deviations)*

	22 Normal Adult Rats	6 Rats with Polymorphonuclears Over 1,000 per Cu. Mm.	5 Rats with Polymorphonuclears Between 500 and 1,000 per Cu. Mm.	5 Rats with Polymorphonuclears Between 100 and 500 per Cu. Mm.	7 Rats with Polymorphonuclears Less Than 100 per Cu. Mm.
Peripheral Blood:					
Hematocrit reading	45.6 ± 3.9	44.7 ± 2.0	40.5 ± 3.9	43.6 ± 1.5	38.6 ± 4.5
White blood cells per cu. mm.	14,750 ± 5,110	9,485 ± 700	8,740 ± 1,350	6,625 ± 1,060	5,400 ± 3,830
Polymorphonuclears per cu. mm.	3,180 ± 1,970	1,690 ± 373	730 ± 153	240 ± 88	50 ± 28
Eosinophils per cu. mm.	240 ± 171	130 ± 14	1 had 30, 4 had 0	14 ± 20	1 had 20, 6 had 0
Lymphocytes per cu. mm.	10,840 ± 4,332	7,780 ± 796	8,020 ± 1,250	6,360 ± 990	5,350 ± 3,820
Normoblasts per cu. mm.	0 (1 rat had 40)	1 had 40, 5 had 0	60 ± 36	20 ± 27	20 ± 24
Proportion of active marrow	.827 ± 0.21	.731 ± .097	.785 ± .065	.722 ± .080	.685 ± .131
Index of Total Quantity in Marrow: *					
Neutrophilic segmented forms and metamyelocytes (index)	34.5 ± 6.2	22.7 ± 8.8	13.6 ± 4.6	11.2 ± 7.4	1.9 ± 1.0
Neutrophilic myelocytes and premyelocytes (index)	9.2 ± 3.1	2.9 ± 1.5	2.4 ± 0.7	3.0 ± 1.8	2.8 ± 0.9
Eosinophilic granulocytes of all types (index)	8.3 ± 5.3	1.6 ± 1.0	2.0 ± 1.3	1.6 ± 1.1	0.9 ± 0.6
Total myeloid cells (index)	52.0 ± 24.8	27.2 ± 9.4	18.0 ± 4.1	15.8 ± 9.9	5.6 ± 2.1
Erythroid cells of all types (index)	30.8 ± 2.5	34.0 ± 9.3	50.7 ± 3.4	49.0 ± 10.2	48.2 ± 13.3
Myeloid-erythroid ratio	1.93 ± 0.93	0.90 ± 0.53	0.36 ± 0.09	0.37 ± 0.31	0.13 ± 0.06
Blast cells of all types (index)	1.9 ± 0.6	0.7 ± 0.4	0.4 ± 0.1	0.5 ± 0.5	1.2 ± 1.2
Lymphocytes of all types (index)	13.3 ± 4.3	8.5 ± 5.1	6.4 ± 6.8	4.0 ± 2.5	6.2 ± 2.7
Miscellaneous cells † (index)	2.0 ± 1.1	3.4 ± 0.9	3.1 ± 1.3	3.2 ± 1.7	7.2 ± 2.4
Weights:					
Rat weight, Gm.		226 ± 23	250 ± 21	224 ± 22	173 ± 40
Cervical lymph nodes, mg.		191 ± 74	150 ± 37	217 ± 59	250 ± 67
Thymus, mg.		260 ± 83	236 ± 104	236 ± 55	126 ± 90
Spleen, mg.		620 ± 175	698 ± 275	740 ± 177	743 ± 420

* See the text for the method of determining this index.
† Reticuloendothelial cells, plasma cells, mast cells, megakaryocytes and unidentified cells are included.

TABLE 3.—*Findings in Individual Rats of a Single Litter*

Days on diet	140	34	116	55	139	93	96
Peripheral Blood:							
Hematocrit reading	49.5	41.7	44.4	42.4	42.3	36.6	38.2
White blood cells per cu. mm.	11,750	14,240	4,320	7,800	9,950	1,350	3,110
Polymorphonuclears per cu. mm.	1,060	870	380	70	50	10	0
Eosinophils per cu. mm.	120	0	0	40	0	0	0
Lymphocytes per cu. mm.	10,570	13,370	3,940	7,690	9,900	1,340	3,110
Normoblasts per cu. mm.	0	15	30	0	0	0	140
Proportion of active marrow	.896	.872	.898	.816	.641	.918	.884
Index of Total Quantity in Marrow: *							
Neutrophilic segmented forms and metamyelocytes (index)	30.4	33.3	7.4	5.0	2.0	2.3	2.8
Neutrophilic myelocytes and premyelocytes (index)	8.8	6.8	5.3	2.9	1.6	4.2	7.6
Eosinophilic granulocytes of all types (index)	5.1	4.3	1.3	1.8	1.5	2.5	0
Total myeloid cells (index)	44.3	44.4	14.0	9.7	5.1	9.0	10.4
Total erythroid cells (index)	26.9	27.6	42.2	55.6	25.1	49.9	6.9
Myeloid-erythroid ratio	1.65	1.62	0.68	0.18	0.20	0.18	1.50
Blast cells of all types (index)	2.4	0.5	2.2	0.6	0.7	1.5	3.3
Lymphocytes of all types (index)	11.7	13.3	20.5	13.2	24.4	21.9	45.6
Miscellaneous cells † (index)	4.3	1.5	11.0	2.4	25.1	9.5	24.0
Weights:							
Rat weight, Gm.	343	292	204	194	258	196	
Cervical lymph nodes, mg.	330	420	260	160	290	310	
Thymus, mg.	190	330	40	30	80	70	
Spleen, mg.	1,100	1,280	1,260	920	1,900	820	

* See the text for the method of determining this index.
† Reticuloendothelial cells, plasma cells, mast cells, megakaryocytes and unidentified cells are included.

in the pronormoblast group. The decrease in cellularity of the marrow was much less than in the group of rats given the experimental diet

Wide variation in the time of onset of the changes in the blood and the marrow was clearly shown in the one litter in which individual mem-

bers were killed at various intervals (table 3). This litter is of interest too in that it demonstrated that the erythroid series in the marrow is depressed in some rats when the deficiency is of long standing. Of interest also in these rats is the striking increase of lymphocytes, plasma cells and reticuloendothelial cells, which in several cases produced hypercellular marrow.

In most rats the lymphoid apparatus showed relatively minor changes. The average weights of the lymph nodes and the spleen, as shown in table 2, are not correlated with the degree of granulocytopenia. The weight of the thymus, however, decreased with progressive granulocytopenia. Study of the individual animal records reveal that when the circulating lymphocytes fell below 2,000 per cubic millimeter, the thymus underwent acute involution, the lymphoid follicles of the spleen and the lymph nodes became atrophic and small lymphocytes largely disappeared from the entire lymphoid apparatus. This lymphoid depletion generally coincided with the onset of marked loss of weight and may be due to acute inanition.

There were no lesions of the liver, the pancreas, the kidneys, the adrenal glands, the heart or the skeletal muscle. Three rats showed pulmonary infections.

Marrow in Rats Showing Granulocytopenia and Anemia.—The peripheral blood of some rats given the succinylsulfathiazole-containing diet from weaning showed leukopenia, granulocytopenia and anemia (table 4). The anemia was of the normochromic normocytic type and was regularly accompanied by moderate numbers of circulating normoblasts and reticulocytes. The marrow of such rats differed little from that of rats showing only leukopenia and granulocytopenia. It was slightly less hypocellular, and there was more depletion of the myeloid series with less depletion of the erythroid series.

TABLE 4.—*Bone Marrow Indexes in Experimental Granulocytopenia and Anemia Before and After "Folic Acid" Treatment (Means and Standard Deviations)*

	10 Rats Given No "Folic Acid"	1 Rat After 2 Days of "Folic Acid" Therapy	6 Rats After 4 Days of "Folic Acid" Therapy	4 Rats After 8 Days of "Folic Acid" Therapy	2 Rats After 12 Days of "Folic Acid" Therapy
Peripheral Blood:					
Hemoglobin, Gm. per 100 cc.	9.7 ± 1.6	.....	8.2 ± 1.5	12.7 ± 2.6	12.6 ± 2.7
Hematocrit reading	27.1 ± 4.0	24.5	25.8 ± 5.1	39.2 ± 6.7	41.5 ± 1.1
Red blood cells per cu. mm. (millions)	4.38 ± 1.38	.....	3.48 ± 0.82	6.42 ± 1.60	6.28 ± 1.66
White blood cells per cu. mm.	2,460 ± 2,372	1,830	6,520 ± 4,500	27,820 ± 7,780	21,325 ± 6,540
Polymorphonuclears per cu. mm.	26 ± 39	610	2,660 ± 3,300	13,740 ± 14,280	4,475 ± 1,380
Eosinophils per cu. mm.	0 ± 0	110	130 ± 150	75 ± 200	130 ± 195
Lymphocytes per cu. mm.	2,434 ± 2,350	1,110	4,460 ± 3,050	14,010 ± 9,530	16,590 ± 4,800
Normoblasts per cu. mm.	765 ± 2,180	2,770	9,280 ± 8,280	13,220 ± 15,780	1,300 ± 1,300
Reticulocytes, per cent	2.9 ± 6.8	.....	31.0 ± 15.7	8.0 ± 3.5	44.0 ± 54.0
Proportion of active marrow	.377 ± .224	.854	.830 ± .108	.933 ± .005	.927 ± .035
Index of Total Quantity in Marrow: *					
Neutrophilic segmented form and metamyelocytes (index)	0.4 ± 0.8	2.5	13.3 ± 12.4	50.0 ± 11.6	39.1 ± 20.5
Neutrophilic myelocytes and premyelocytes (index)	1.9 ± 2.5	7.3	12.5 ± 8.7	8.8 ± 5.4	2.8 ± 6.0
Eosinophilic granulocytes of all types (index)	0.5 ± 0.8	3.8	1.9 ± 1.4	1.4 ± 0.9	2.8 ± 3.3
Total myeloid cells (index)	2.8 ± 3.3	13.6	27.7 ± 16.4	60.2 ± 10.0	44.7 ± 16.5
Erythroid cells of all types (index)	23.3 ± 17.5	62.2	50.1 ± 11.0	12.9 ± 6.5	38.3 ± 11.3
Myeloid-erythroid ratio	0.14 ± 0.44	0.22	0.39 ± 0.58	5.83 ± 3.58	1.28 ± 0.81
Blast cells of all types (index)	0.4 ± 0.3	3.1	0.9 ± 0.5	1.9 ± 2.9	0.7 ± 0.2
Lymphocytes of all types (index)	8.3 ± 4.4	2.2	2.9 ± 2.0	11.6 ± 11.6	6.4 ± 0.1
Miscellaneous cells † (index)	3.0 ± 3.6	4.3	1.4 ± 1.1	4.4 ± 2.7	2.8 ± 1.9

* See the text for the method of determining this index.
† Reticuloendothelial cells, plasma cells, mast cells, megakaryocytes and unidentified cells are included.

Three rats showed bacteremia, and 3 showed pulmonary infections. There was lymphoid exhaustion of the spleen as well as absence of splenic hemopoiesis. Necrosis and calcification of skeletal muscle were common. No lesions were noted in the other viscera. There was no definite evidence of bartonella infection, although a few suspicious inclusions were seen in erythrocytes in 2 rats.

Effect of "Folic Acid" on the Marrow of Rats Showing Granulocytopenia and Anemia.—Treatment with "folic acid" evoked marked response. The circulating red and white cells all increased. Large numbers of normoblasts and reticulocytes appeared. In the marrow the erythroid series showed the most marked response during the

first four days. After eight days the myeloid elements had increased markedly at the expense of the erythroid elements. In twelve days the marrow was almost entirely normal except for some excess of the more mature forms of the myeloid series.

COMMENT

In these experimental dyscrasias there is no maturation arrest in the usual sense with a piling up of large numbers of immature cells. From the very beginning, even while the peripheral blood is within low normal limits, there is hypoplasia of the marrow. The myelocytes and the promyelocytes decrease more rapidly than the metamyelocytes and the segmented forms at first. In individual rats the metamyelocytes and the

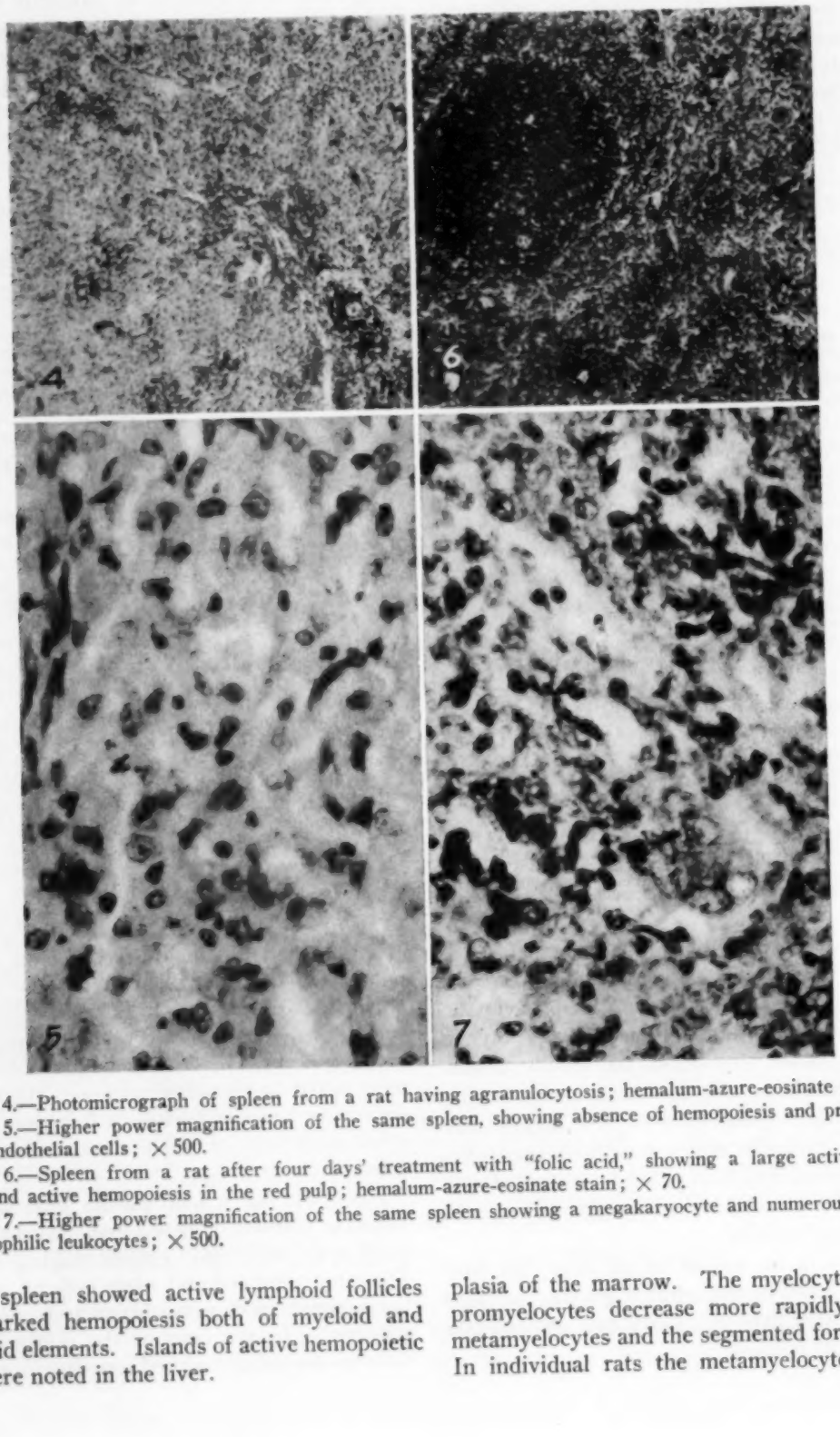

Fig. 4.—Photomicrograph of spleen from a rat having agranulocytosis; hemalum-azure-eosinate stain; × 70.

Fig. 5.—Higher power magnification of the same spleen, showing absence of hemopoiesis and prominence of reticuloendothelial cells; × 500.

Fig. 6.—Spleen from a rat after four days' treatment with "folic acid," showing a large active lymphoid follicle and active hemopoiesis in the red pulp; hemalum-azure-eosinate stain; × 70.

Fig. 7.—Higher power magnification of the same spleen showing a megakaryocyte and numerous precursors of neutrophilic leukocytes; × 500.

The spleen showed active lymphoid follicles and marked hemopoiesis both of myeloid and erythroid elements. Islands of active hemopoietic cells were noted in the liver.

segmented forms disappear from the marrow, but usually a few myelocytes and premyelocytes persist. There is hypoplasia of the erythroid series, megakaryocytes and blasts. Reticuloendothelial cells, plasma cells and occasionally lymphocytes increase somewhat.

The presence or the absence of anemia cannot be correlated with the changes in the marrow. There is little difference between the marrow of a rat showing only granulocytopenia and that of a rat showing granulocytopenia and anemia. Since the erythroid series of the marrow is depleted in all cases, it would appear that the presence or the absence of anemia observed in these studies depends on something in addition to the state of the marrow. The work of Kornberg, Tabor and Sebrell [8] shows clearly that latent anemia can be demonstrated by repeated bleeding and measurement of the rate of erythrocyte regeneration.

The lymphopenia is not particularly striking until the onset of loss of weight. Histologic study of the lymphatic apparatus indicates that the profound lymphoid exhaustion occurs largely after the animal has practically stopped eating. This suggests that inanition may play an important role in the lymphopenia.

The prompt spectacular response of the marrow and the spleen to "folic acid" might be expected from the response seen in the peripheral blood.

The mechanism whereby "folic acid" is required for normal hemopoiesis cannot be deduced from a study of this sort. It is evident, however, that it is required for survival and multiplication of the immature myeloid and erythroid cells. Gross, Axelrod and Bosse [9] in their studies found considerable damage of the liver and observed that "folic acid" prevented the hepatic lesions and perhaps produced some regeneration in the damaged liver. They suggested the possibility that the "folic acid" is essential for normal functioning of the liver, which in turn produces something which is essential for hemopoiesis. Our results do not support this theory. We noted severe hypoplasia of marrow with leukopenia, agranulocytosis and anemia in rats whose livers appeared entirely normal. Histologically demonstrable damage of the liver is not a prerequisite for involvement of the marrow. We cannot exclude the possibility, however, that hepatic function may have been impaired despite the absence of histologic changes.

It would be interesting to compare histologically this experimental granulocytopenia and agranulocytosis with the like disorders in man. Unfortunately, this is scarcely possible, since most clinical studies have not been made in a quantitative fashion. The usual postmortem studies of human marrow are made from sections of nitric acid–decalcified rib or sterum, in which accurate delineation of cell types is difficult. The usual antemortem studies of specimens of sternal marrow obtained by aspiration, a procedure yielding good smears in capable hands, are not to be relied on for measurement of the amount of active marrow. Observations made with such technical limitations must be accepted with reservation. Furthermore, the comparison is not valid because of the variety of exciting causes of agranulocytosis in man. The histologic conditions reported in the literature vary from myeloid aplasia or even panmyelophthisis to myeloid hyperplasia with large numbers of myelocytes and premyelocytes or even aleukemic myelogenous leukemia. Several attempts to integrate these conflicting reports have been made. Perhaps the best of these is found in the work of Rohr.[10] He suggested that the basic defect is a maturation disturbance of leukopoiesis, often as a shock phenomenon (in response to exogenous substances to which the patient is hypersensitive). The resulting myelogram depends on the stage of the disease and the severity of the shock. Thus a mild shock may inhibit final maturation, with granulocytopenia and peripheral "left shift" and a myelogram with normal cellularity but with a decrease in segmented forms and with a normal amount or an increase of younger forms. More severe shock or repeated shock may result in myeloid hypoplasia which in the most severe forms results in myeloid aplasia. He pointed out the frequent concurrent lymphopenia and thrombopenia and less frequently anemia, and his case reports show evidences of involvement of erythroid and megakaryocytic series in the marrow. In the regenerative phases he observed hyperplasia with preponderance of the younger myeloid elements. Superficially, then, there is considerable similarity between the experimental observations reported here and the clinical observations in human agranulocytosis of certain types. No conclusions appear to be justified from this superficial similarity.

From the pathologist's point of view, the chief importance of experimental granulocytopenia and agranulocytosis and anemia of this type lies in its unique adaptability to research. Further studies may serve to clarify many of the perplexing physiologic and pathologic reactions of the marrow.

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SUMMARY

Leukopenia, granulocytopenia and, less frequently, anemia develop in rats given succinylsulfathiazole in purified diets. L. casei factor ("folic acid" or vitamin B_c) corrects or prevents these dyscrasias. The involved marrow is hypocellular and shows general depletion, especially of the myeloid series. The erythroid series is usually depleted even though no anemia may be found. The response of the marrow to "folic acid" is a rapid regeneration with spectacular myeloid proliferation. When anemia is also present, the erythroid series becomes hyperplastic and later is crowded out by myeloid hyperplasia. The marrow returns to normal after temporary overcompensation.

EXPERIMENTAL ATHEROSCLEROSIS

VIII. THE EFFECT OF FEEDING OLIVE OIL ON THE ABSORPTION AND DEPOSITION OF CHOLESTEROL

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It has been shown frequently that when cholesterol is added to the diet of the rabbit the concentration of this lipid in various tissues of the body, particularly the blood, the liver and the vascular walls, is increased. Versé [1] observed that the blood cholesterol reached high levels much more quickly and with smaller daily feedings of cholesterol when this was dissolved in oil and fed by stomach tube. Weinhouse [2] compared the results of Weinhouse and Hirsch [3] with those of Page and Bernhard [4] and demonstrated that the feeding of cholesterol in oil apparently enhanced the absorption of the cholesterol. The ingestion of 1 Gm. of cholesterol per day (Weinhouse and Hirsch) produced approximately the same values for blood cholesterol as the daily feeding of 0.2 Gm. of cholesterol in oil (Page and Bernhard).

Chanutin and Ludewig [5] found that supplementing a cholesterol diet with high concentrations of fat or carbohydrate influenced lipid metabolism in the rat. Oddly enough, they demonstrated that carbohydrate might accelerate, and fat inhibit, the deposition of cholesterol in the liver of the rat. Cook [6] showed, however, that cholesterol was absorbed readily and fatty livers were produced in the rat only when fat was present in the diet. He found guinea pigs rather susceptible to cholesterol when this was added to a stock diet which contained little fat. In a later paper Cook [7] showed that increasing the fat content of a diet containing 2 per cent cholesterol by 15 per cent to 30 per cent did not increase the absorption of cholesterol. On the other hand, Loizides [8] showed that graded increments in the concentration of fat in the diet of rats fed 2 per cent cholesterol caused progressive increases in the cholesterol ester content of the liver.

In studies on rats, guinea pigs and rabbits Cook and McCullagh [9] showed that the feeding of cholesterol in oil increased the blood cholesterol and the hepatic lipids of the rat; in the guinea pig, the blood cholesterol alone was augmented; in the rabbit the blood, the liver and the aorta showed high concentrations of cholesterol.

In the present study, the effect of adding cholesterol with and without fat (olive oil) to the basal diet on the cholesterol content of the blood, the liver and the aorta of the rabbit was reinvestigated. It appeared important to pursue this problem further in view of the conflicting reports in the literature.

MATERIAL AND METHODS

Thirty-six female rabbits, 3 to 5 months old, were used. Each animal received 1 Gm. of cholesterol, mixed with the chow,[10] three times a week; to the basal diet of 18 of these animals 2.5 cc. of olive oil was added and mixed with the food on the days of the cholesterol feeding.

At the end of the experimental period (twelve weeks) the rabbits were killed. The procedure used for killing the animals, removing the aorta and determining its cholesterol content has been described elsewhere.[11] Total and ester cholesterol in whole blood were determined at intervals of two weeks by a modified Bloor method.[12] The wet liver was analyzed for cholesterol by the procedure of Bloor and Knudson.[13]

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RESULTS

Tables 1 and 2 detail the results obtained. The rabbits fed the chow-cholesterol mixture showed an eightfold increase in the total cholesterol of the whole blood at the tenth week; the animals fed the chow–cholesterol–olive oil rations attained slightly higher levels at about the same time. Esterified cholesterol increased in absolute 1,129 mg. per hundred grams in the rabbits fed cholesterol alone. Olive oil feeding increased the average cholesterol content of the aorta by more than 1,000 mg. per hundred grams of dry weight (4,115 mg. per hundred grams in the olive oil–cholesterol group, compared with 3,088 mg. per hundred grams in the cholesterol-fed animals).

TABLE 1.—*The Effect of Feeding 1 Gm. of Cholesterol Three Times Weekly on the Cholesterol Content of the Blood, the Liver and the Aorta of the Rabbit*

	Cholesterol in Whole Blood														Cholesterol in Liver, Mg. per 100 Gm.	Cholesterol in Aorta, Mg. per 100 Gm.
	Total, Mg. per 100 Cc.							Ester, per Cent of Total								
Rabbit	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks	Wet Wt.	Dry Wt.
22	100	175	344	361	658	962	872	..	..	..	..	..	..	..	699	2,641
23	130	303	250	217	258	242	596	..	..	..	..	..	..	..	452	645
24	110	383	559	938	815	890	625	21	54	46	51	50	48	40	943	9,615
25	105	222	391	326	417	658	658	17	39	47	23	42	50	30	1,009	1,645
26	137	289	500	596	682	765	833	25	37	44	40	41	42	41	642	2,840
27	99	205	347	417	750	1,103	1,103	34	54	44	47	41	41	51	509	3,375
28	127	341	178	154	278	260	266	22	55	53	47	50	36	28	442	934
29	90	371	625	670	625	987	1,136	25	41	62	38	50	47	36	723	3,538
30	85	257	266	421	708	551	...	22	49	66	47	51	29	..	1,116	1,156
42	98	118	95	217	391	586	586	15	19	..	41	55	..	59	1,473	682
43	96	83	332	196	326	596	615	12	44	..	43	56	55	47	847	695
70	64	187	521	725	893	1,108	852	9	39	64	63	70	55	44	1,961	5,234
71	64	199	68	162	250	198	278	14	32	31	59	44	30	24	961	505
72	92	300	636	852	781	938	962	11	55	57	63	56	52	41	1,700	6,000
73	69	175	481	514	577	725	750	14	21	71	69	55	57	50	1,280	6,012
74	66	201	205	417	313	403	347	13	38	53	71	45	43	50	1,013	1,551
75	64	625	798	938	1,014	987	798	9	57	64	71	19	60	35	2,083	3,321
76	63	682	833	815	962	1,139	1,071	17	55	57	57	19	45	43	2,466	5,208
Average	92	284	413	496	594	727	726	18	43	54	52	46	46	41	1,129	3,088

TABLE 2.—*The Effect of Feeding 1 Gm. of Cholesterol and 2.5 cc. of Olive Oil Three Times Weekly on the Cholesterol Content of the Blood, the Liver and the Aorta of the Rabbit*

	Cholesterol in Whole Blood														Cholesterol in Liver, Mg. per 100 Gm.	Cholesterol in Aorta, Mg. per 100 Gm.
	Total, Mg. per 100 Cc.							Ester, per Cent of Total								
Rabbit	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks	0 Weeks	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	12 Weeks	Wet Wt.	Dry Wt.
50	192	450	750	938	...	708	815	36	61	49	41	..	40	51	2,268	2,900
51	144	371	682	987	...	1,293	1,339	38	47	48	38	..	52	56	3,232	12,931
53	96	368	750	1,500	...	1,705	1,563	21	34	47	35	..	37	54	2,480	12,019
39	109	200	379	170	158	625	625	25	37	32	55	53	..	78	2,429	1,117
40	108	100	245	140	341	487	750	16	40	21	47	59	51	36	1,171	900
41	80	259	132	257	250	284	...	24	28	32	..	36	46	..	1,200	435
86	68	586	586	694	750	1,014	833	13	49	78	62	74	57	..	3,255	10,085
87	71	335	987	915	893	1,631	1,339	17	40	63	67	63	57	34	4,974	5,305
88	128	217	183	326	452	658	670	32	41	60	61	76	52	34	1,442	1,600
89	75	150	507	403	252	300	250	11	50	62	62	..	36	37	762	1,807
90	57	250	586	833	1,072	1,380	938	14	60	66	62	70	71	41	1,359	5,814
91	88	149	586	577	551	1,000	577	12	33	75	57	66	42	46	1,832	3,030
92	81	223	217	364	309	536	559	11	47	89	60	45	53	39	1,977	1,113
93	80	181	282	514	725	725	596	12	30	60	55	28	58	47	1,838	5,555
94	82	481	446	521	750	...	...	13	63	..	56	48	..	..	3,068	1,900
95	82	280	293	320	568	1,139	1,014	13	32	70	62	55	45	49	3,000	1,783
96	64	375	487	586	586	798	...	14	50	..	64	53	19	..	1,959	1,671
97	68	278	586	708	795	...	...	14	61	67	73	48	..	..	2,441	.....
Average	93	292	482	598	569	893	848	19	45	57	56	55	48	46	2,259	4,115

quantities and in the percentage of the total whole blood cholesterol in both groups to about the same extent.

The addition of olive oil to the chow-cholesterol mixture had a decided effect on the amount of this sterol deposited in the liver and the aorta. The average cholesterol content of the liver in the olive oil–cholesterol group was 2,259 mg. per hundred grams of wet weight, compared with

COMMENT

It has been demonstrated frequently that cholesterol when fed in combination with low fat diets is absorbed readily and rises to high levels in the blood.[14] Since this sterol is soluble in

14. Turner, K. B.: J. Exper. Med. **58**:115, 1933. Turner, K. B., and Bidwell, E. H.: ibid. **62**:721, 1935. Meeker, D. R.; Kesten, H. D., and Jobling, J. W.: Arch. Path. **20**:337, 1935. Huber, M. J.; Broun, G. O.,

(Footnote continued on next page)

solutions of bile salts, particularly in those containing sodium desoxycholate (Bashour and Bauman [15]), it may be assumed that the presence of adequate quantities of bile in the intestinal tract determines the degree of absorption of cholesterol. Of interest is the fact that Spanner and Bauman [16] showed that the addition of sodium oleate to sodium desoxycholate in vitro almost doubled its solvent properties for cholesterol. The feeding of olive oil,[17] therefore, should enhance the absorption of cholesterol from the intestinal tract. As gaged from blood cholesterol levels in the present experiments, olive oil augmented the absorption of cholesterol only slightly. It is conceivable that the amount of olive oil used (2.5 cc. three times weekly) was insufficient to show this effect more strikingly.

CONCLUSIONS

The feeding of olive oil with the cholesterol added to the diet produced slightly higher blood cholesterol levels in rabbits than did the feeding of cholesterol alone.

The addition of olive oil to the cholesterol ration increased appreciably the deposition of cholesterol in the liver and the aorta.

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and Casey, A. E.: Proc. Soc. Exper. Biol. & Med. **37**: 441, 1937. Bruger and Fitz.[11] Member, S.; Bruger, M., and Oppenheim, E.: Arch. Path. **38**:210, 1944.

15. Bashour, J. T., and Bauman, L.: J. Biol. Chem. **121**: 1, 1937.

16. Spanner, G. O., and Bauman, L.: J. Biol. Chem. **98**:181, 1932.

17. Olive oil contains 82 per cent oleic acid, 8 per cent palmitic acid, 5 per cent linoleic acid, 2 per cent stearic acid, 1 per cent palmitoleic acid and traces of myristic and arachidic acids (Everett, M. R.: Medical Biochemistry, New York, Paul B. Hoeber, Inc., 1942, p. 175).

LOEFFLER'S SYNDROME

REPORT OF A CASE WITH PATHOLOGIC EXAMINATION OF THE LUNGS

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In 1932 and again in 1936 Löffler [1] reported a syndrome characterized by transitory pulmonary lesions, a remarkable increase of eosinophilic leukocytes in the blood and a mild clinical course which was in striking contrast to the extensive pulmonary changes observed in the roentgenograms. Although there have been numerous clinical and roentgenologic studies of this symptom complex, the anatomic basis of the disorder has not been well understood. Various observers have interpreted the lesions shown in the roentgenograms as edema, infarcts and pneumonia. Since pathologic observations on this condition have been limited because of the relative benignity of the disease, it was considered worth while to report a case in which the lungs were obtained for examination.

REPORT OF CASE

A woman of 59 years was referred to Dr. D. O. N. Lindberg, of Buena Vista Sanatorium, by Dr. E. C. Bayley, of Lake City, Minn. Dr. Bayley had observed abnormal and atypical roentgenographic markings in association with a symptomatic nonspasmodic "asthma" of seven years' duration.

The patient was admitted to the sanatorium on July 3, 1944 for observation to rule out the possibility of pulmonary tuberculosis. She complained of severe cough, breathlessness and anorexia. She weighed 135 pounds (61.2 Kg.) although her usual weight had been 196 pounds (88.9 Kg.). Physical examination revealed extensive varicose veins in both legs, with pigmentation of the skin. There were a postoperative ventral hernia and extensive scars of the right forearm, the result of a previous burn. The blood pressure was within normal limits, and the thorax and the abdomen revealed nothing remarkable.

In roentgenograms of the thorax that had been taken on June 24, 1944 there were shadows of varying size, shape and density in both upper lobes. They were not suggestive of tuberculosis (fig. 1). A Mantoux test with 1.0 mg. of old tuberculin was interpreted as giving a negative result. On routine examination of the blood the erythrocytes numbered 4,600,000 and the leukocytes 12,550 per cubic millimeter. The differential count revealed 40 per cent neutrophils, 35 per cent eosinophils, 24 per cent lymphocytes and 1 per cent monocytes. The concentration of hemoglobin was 14.5 Gm. per hundred cubic centimeters of blood. The sedimentation rate (Cutler method) was 25 mm. at the end of the first hour. The Kahn flocculation test of serum for syphilis showed a negative reaction. Examination of the sputum by means of appropriately stained smears and concentration methods failed repeatedly to demonstrate infection. Attempts to culture tubercle bacilli were without success. Monilia albicans was isolated in pure culture and proved nonpathogenic in mice. No other yeasts or fungi were found. Direct smears of the sputum revealed epithelial cells, leukocytes, cocci and bacilli.

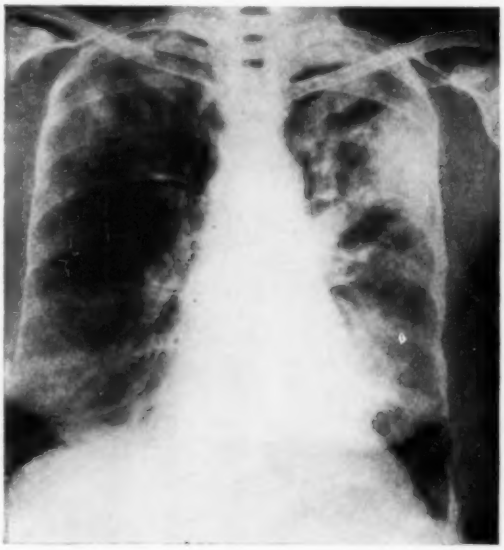

Fig. 1.—Roentgenogram of the thorax taken June 24, 1944.

During the patient's course in the sanatorium the temperature varied between 99 and 99.6 F. and the pulse between 72 and 80 beats per minute. Sodium iodide was administered intravenously in doses of 2 Gm.

From the Section on Pathologic Anatomy, Mayo Clinic.

1. Löffler, W.: Beitr. z. Klin. d. Tuberk. **79**:368, 1932; Schweiz. med. Wchnschr. **66**:1069, 1936.

on three consecutive days, July 11 to 13 inclusive. There were slight elevations of the temperature on the days when sodium iodide was administered, but these disappeared within seventy-two hours following the last of the three injections. Scattered painful pustular eruptions of the skin occurred after each administration of sodium iodide. The sputum increased in amount from 3 Gm. to 55 Gm. by July 18. Roentgenograms of the thorax were examined on July 30 and August 3. There was marked bilateral regression of the lesions (fig. 2*a* and *b*). It was concluded that this definitely ruled out the possibility of neoplasm of the lung as well as tuberculosis. By August 3 the amount of sputum had decreased to 6 Gm., and 2 Gm. of sodium iodide was again given intravenously. Pulse and temperature reactions similar to those observed at the previous injections occurred. The sputum increased in amount, varying between 18 and 28 Gm. The patient was dismissed on Sept. 11, 1944. During her stay at the sanatorium, her weight had increased from 135 to 140½ pounds (61.2 to 63.7 Kg.), the leukocyte count had decreased

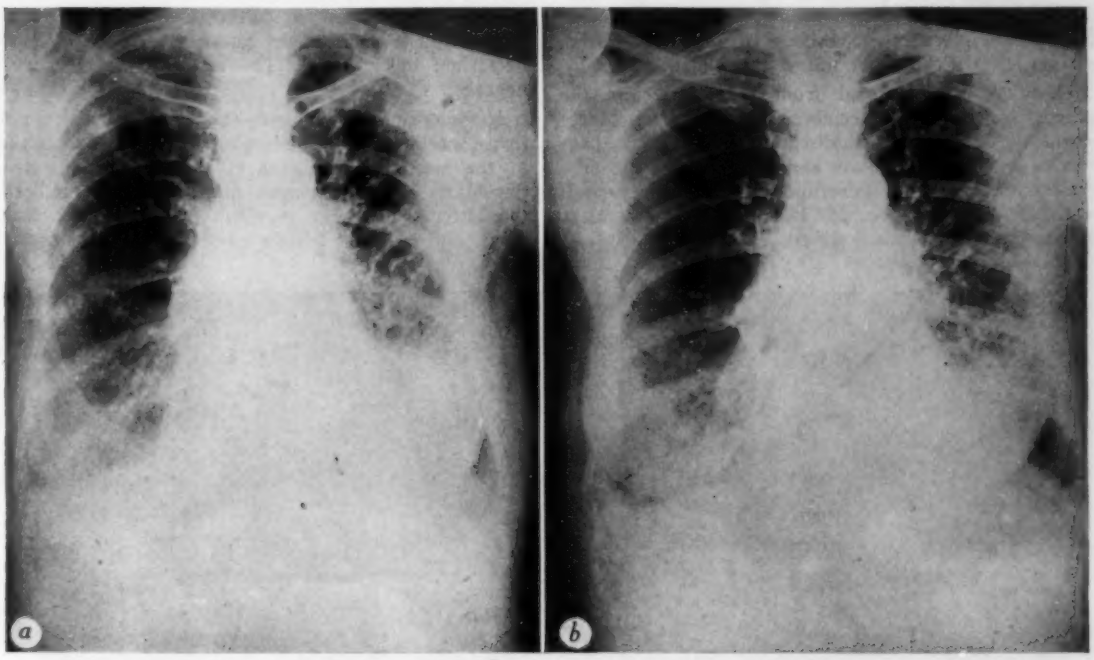

Fig. 2.—Roentgenograms of the thorax taken (*a*) July 30 and (*b*) Aug. 3, 1944. Note regression of the lesions.

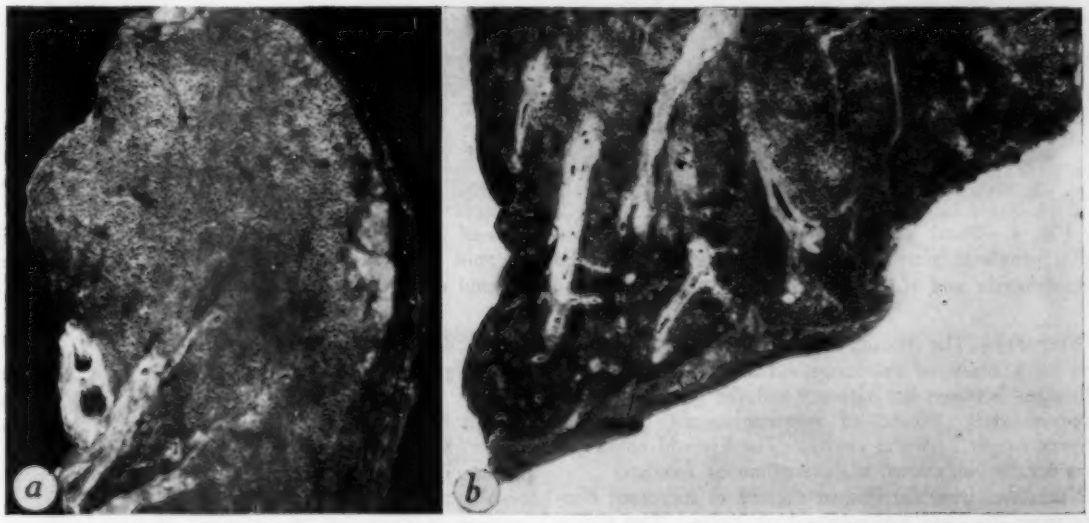

Fig. 3.—(*a*) Upper lobe of the left lung, showing firm nodular lesions of varying size and shape and emphysema. (*b*) Lower lobe of the same lung, showing areas of consolidation and focal granulomatous lesions.

from 12,550 to 10,800 per cubic millimeter, and the percentage of eosinophils was reduced from 35 to 22. The sedimentation rate had diminished from 25 mm. to 20 mm. The roentgenograms of the thorax revealed the presence of only a few residual areas of abnormal density. The patient was advised to have the intravenous therapy with sodium iodide continued and was instructed to have roentgenograms of the thorax made at monthly intervals. She felt so much improved, however, that she did not follow these instructions.

Following her dismissal from the sanatorium the patient felt well until December 21, when she suddenly became ill with substernal discomfort and severe, progressive dyspnea. Cyanosis appeared and became progressively worse, and generalized edema supervened. Death occurred on Dec. 26, 1944, the fifth day after the onset of her terminal illness.

Histologic Examination. — Sections taken from the firm, scarlike regions revealed extensive replacement of the normal parenchyma of the lung by masses of fibroblasts and collagenous fibers. In the meshes of the latter were large numbers of eosinophilic leukocytes, plasma cells, lymphocytes and some giant cells.

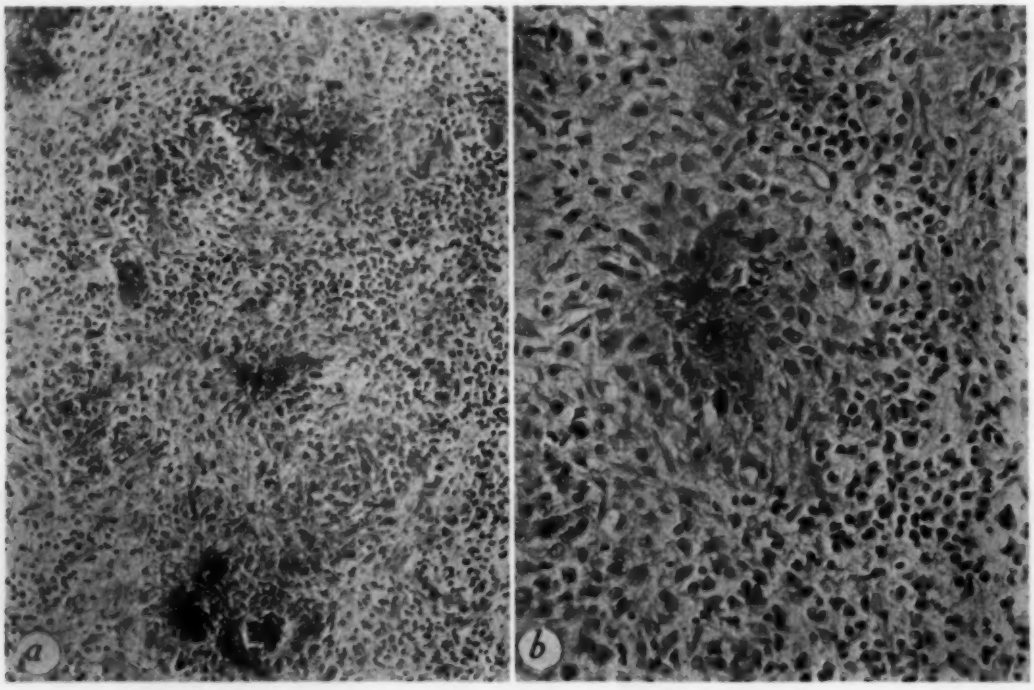

Fig. 4.—Focal granulomatous lesions: (*a*) Central areas of acidophilic granular necrotic tissue surrounded by a proximal zone of epithelioid cells and a peripheral zone of eosinophilic leukocytes and plasma cells (hematoxylin and eosin; × 105). (*b*) Same (hematoxylin and eosin; × 275).

Necropsy.—The postmortem examination was limited to a study of the lungs. There were numerous adhesions between the visceral and the parietal pleura. Approximately 300 cc. of serosanguineous fluid was present in each pleural cavity. The bronchi contained considerable tenacious mucosanguineous exudate.

Numerous irregular-shaped regions of increased density were scattered throughout both lungs. The pleura was thickened over these regions, and adhesions were frequently present. The cut section revealed these areas to be of varying size and shape (fig. 3 *a* and *b*). The largest measured roughly 5.0 cm. in its greatest extent, while the smallest were only a few millimeters in diameter. Some of the latter resembled tubercles. The nodular regions were grayish yellow and firm to palpation, and no exudate could be expressed from them. In the lower lobes there were larger, dark red, consolidated regions at the bases which exuded serum and blood. The bronchi contained mucus, and the walls of some of them were greatly thickened. The alveoli appeared dilated, especially in the upper lobes. Portions of the nodular and the consolidated regions were removed with sterile instruments and referred to Dr. W. H. Feldman for culture and guinea pig inoculation with a view to the possible presence of Mycobacterium tuberculosis. The results were reported as negative.

These cells were found in focal collections and also diffusely distributed throughout the new connective tissue. The appearance was that of an unusual form of organized pneumonia in which there was an abnormally large number of eosinophilic leukocytes. In some portions of the pneumonic process there were also focal, tubercle-like lesions (fig. 4 *a* and *b*). The centers of these lesions consisted of acidophilic granular necrotic material. Mononuclear cells and fibroblasts could sometimes be observed in between the clumps of acidophilic granular necrotic debris. Eosinophilic granules could be detected in the clumps of granular material. Large histiocytes, epithelioid cells and fibroblasts surrounded the central portion of the lesion. These cells were usually arranged radially. An outer zone of eosinophilic leukocytes, plasma cells

and lymphocytes was present. Multinucleated giant cells of variable shape and nuclear arrangement were observed occasionally in the tubercle-like lesions. The latter did not have the typical appearance of the tubercles of tuberculosis. A superficial resemblance to the granulomatous lesion of rheumatic fever was discernible. The walls of the arteries and arterioles were moderately thickened. The capillaries were dilated, and hemorrhages were common. There was a severe periarterial and pericapillary inflammatory process in which eosinophilic leukocytes and plasma cells were the principal cellular elements. Actual necrosis of the arterial and venous walls was occasionally observed (fig. 5 *a* and *b*). In the region surrounding the organized pneumonia the alveolar walls were much thickened by a cellular exudate consisting largely of eosinophilic leukocytes, plasma cells and lymphocytes. This portion had the appearance of interstitial pneumonia. Moderate emphysema was present.

In sections of the lower lobes a more recent inflammatory process was evident. The alveoli contained serum, red cells and fibrin in addition to the cellular elements observed in the upper lobe. Large phagocytic cells were numerous and were found to contain vacuoles and pigment. Beginning organization of intra-alveolar fibrin was occasionally observed. The same vascular changes were observed as were present in the upper lobe. The tubercle-like lesions were frequently present. The bronchioles were filled with mucus and desquamated epithelial cells. Thickening and hyalinization of the basement membrane were prominent. There was no evidence of hypertrophy of the musculature but the walls were infiltrated with eosinophilic leukocytes and plasma cells. Interstitial pneumonia was also present in some regions. Moderate to severe emphysema was observed. Arteriolar sclerosis was graded 3 (on the basis of 1 to 4, in which 1 designates the least and 4 the greatest amount of sclerosis).

Sections of the upper and the lower lobes were appropriately stained for bacilli of tuberculosis, but no organisms could be found. No micro-organisms could be detected in sections stained by the Brown-Gram method.

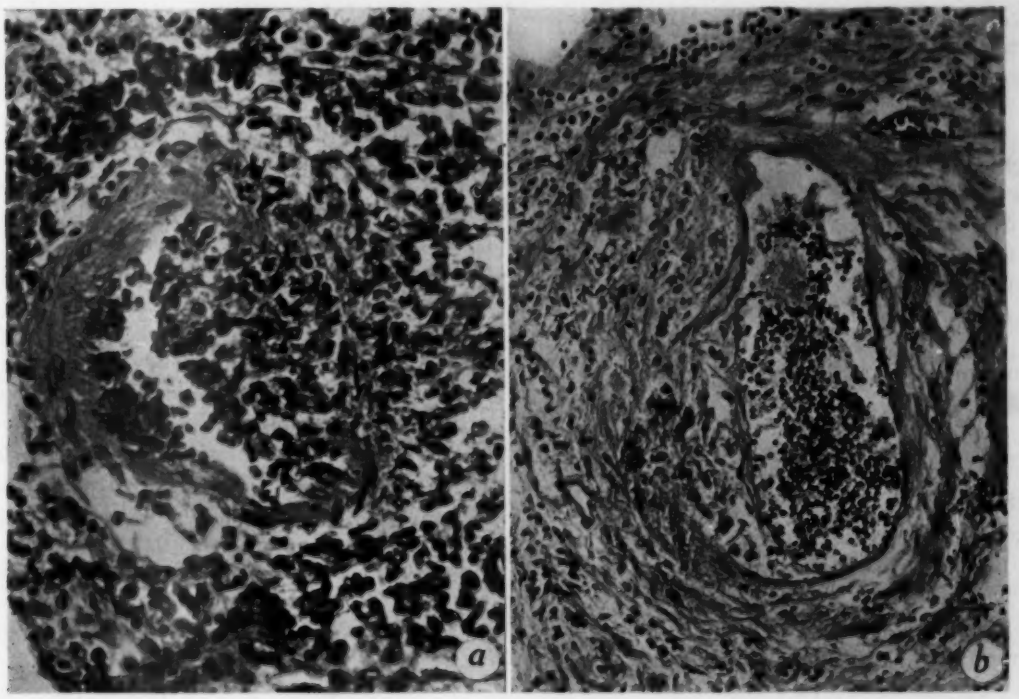

Fig. 5.—Vascular lesions: (*a*) Necrotizing arteriolitis (hematoxylin and eosin; × 275). (*b*) Necrotizing phlebitis (hematoxylin and eosin; × 160).

COMMENT

As Löffler himself predicted, there has been considerable delay in uncovering the pathologic anatomic basis of the syndrome bearing his name. This is due to the transitory character of the pulmonary lesions and the relative benignity of the disease. Meyenburg [2] was the first pathologist fortunate enough to obtain material for study. He reported 4 cases in which pulmonary lesions which he believed represented the anatomic substrate of Löffler's syndrome were observed incidentally at necropsy. Three of the patients had been in apparently good health and died accidentally while in military service. The fourth patient died of tetanus. The lesions that Meyenburg described were essentially those of lobular pneumonia in which the cellular portion

2. von Meyenburg, H.: Schweiz. med. Wchnschr. **72**:809, 1942.

of the exudate consisted largely of eosinophilic leukocytes (70 to 100 per cent). Giant cells were observed fairly constantly. Broch [3] reported a case of Löffler's syndrome in which death was thought to have been the result of ventricular fibrillation following the use of an epinephrine hydrochloride spray. Histologic examination of the pulmonary lesions in his case revealed thickening of the interstitial tissue with abundant lymphocytic and plasma cell infiltration. In some parts, the structure of the lung was obliterated, and the alveoli were filled with lymphocytes, plasma cells and eosinophilic leukocytes. Some of the bronchi were distended and filled with mucus, exfoliated epithelium and the same forms of leukocytes. Around some branches of the bronchi there was intense leukocytic infiltration. There was a remarkably great and widespread eosinophilia. In a recent paper entitled "Vascular Allergy" Harkavy [4] reported the results of a study of a special group of 16 cases of bronchial asthma, in 4 of which necropsy was performed. He stated that the fugitive pulmonary infiltrations referred to by some as Löffler's pneumonia were found to be due to congestion, edema and thrombi in small vessels in 1 case and to infiltration of the interalveolar septums with eosinophils, polymorphonuclears and lymphocytic cells, plus edema, in 2 other cases.

In the present case the most significant features aside from the predominance of eosinophilic leukocytes in the pneumonic exudate were the character of the bronchial lesions, the advanced organization of the pneumonic exudate, the granulomatous character of the focal lesions and the presence of vascular lesions. The bronchial lesions in our case were similar to those observed in bronchial asthma. Hypertrophy of the muscle of the bronchial wall was not pronounced, but the predominantly eosinophilic exudate infiltrating all portions of the wall, the marked hyalinization and thickening of the basement membrane of the epithelium and the presence of large quantities of mucus in the cells and lumens were all typical of bronchial asthma. In this connection it is interesting to note that Meyenburg described eosinophilic bronchitis and bronchiolitis in 2 of his cases. The advanced organization of the pneumonic exudate and the granulomatous character of the focal lesions constitute overwhelming evidence that the pulmonary lesions are not "transitory" in all cases. The fact that the lesions in the lungs may persist for long periods has been confirmed also by Léon-Kindberg, Adida and Rosenthal,[5] Löhr,[6] Kartagener,[6] Meyenburg [2] and Harkavy.[4] The marked clearing of the pulmonary lesions demonstrated by the roentgenographic studies in our case suggests that regression of the lesions does occur but that this does not indicate complete resolution.

The focal granulomatous lesions were extremely interesting. Histologically, they were superficially suggestive of both tuberculous and rheumatic lesions but not typical of either. Fibrinoid swelling and necrosis of collagen were prominent features of these lesions and suggested an allergic basis. This concept was further strengthened by the large numbers of eosinophilic leukocytes in the peripheral portion of the lesion. Somewhat similar lesions have been described by Meyenburg (his case 2).

The vascular lesions were extensive and remarkable. The most constant change observed consisted of hypertrophy of the media and thickening of the intima with consequent narrowing of the lumens. The arterioles and the small and medium-sized arteries were involved. This change was not remarkable since it is frequently observed in cases of pulmonary emphysema. The most striking change, however, was that of necrotizing arteritis and arteriolitis which histologically appeared quite similar to the change observed in periarteritis nodosa. It also suggests that there is an underlying allergic basis in Löffler's syndrome. The thesis that vascular lesions of this type are on an allergic basis has received considerable support in recent years from the works of Gruber,[7] Klinge,[8] Clark and Kaplan,[9] Rich [10] and Rich and Gregory.[11]

In this connection it is interesting that Wilson and Alexander [12] have recently found in a review of 300 cases from the literature that 18 per cent of patients who had periarteritis nodosa had bronchial asthma. Harkavy found arterial

3. Broch, O. J.: Acta med. Scandinav. **113**:311, 1943.

4. Harkavy, J.: J. Allergy **14**:507, 1943.

5. Léon-Kindberg, M.; Adida, P., and Rosenthal, L.: Presse méd. **1**:277, 1940.

6. Löhr, cited by von Meyenburg.[2]

7. Gruber, G. B.: Virchows Arch. f. path. Anat. **258**:441, 1925.

8. Klinge, F., cited by Wilson and Alexander.[12]

9. Clark, E., and Kaplan, B. I.: Arch. Path. **24**: 458, 1937.

10. Rich, A. R.: Bull. Johns Hopkins Hosp. **71**: 123, 1942.

11. Rich, A. R., and Gregory, J. E.: Bull. Johns Hopkins Hosp. **72**:65, 1943.

12. Wilson, K. S., and Alexander, H. L.: J. Lab. & Clin. Med. **30**:195, 1945.

lesions varying from simple thickening of the intima to acute necrotizing arteritis and endarteritis obliterans in the 4 cases of bronchial asthma studied by him.

Although the etiologic factors concerned in this disease are uncertain, most authorities describe the syndrome as an allergic response of the pulmonary tissue that may be induced by various allergens. The allergenic agents described in the reported cases are extremely varied and include Mycobacterium tuberculosis and other bacteria, larvae of Ascaris lumbricoides, Endamoeba histolytica and pollens of various types. None of these agents could be identified in our case.

SUMMARY

The outstanding histologic features of the pulmonary lesions in a case of Löffler's syndrome were the large numbers of eosinophilic leukocytes in the pneumonic exudate, the advanced organization of the exudate with fibrosis and giant cells, the presence of peculiar granulomatous lesions and the occurrence of necrotizing arteritis and phlebitis.

Case Reports

PRIMARY CARCINOMA OF THE LIVER

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CHICAGO

Of 1,817 consecutive autopsies in Freedmen's Hospital, Washington, D. C., covering a period of fifteen years, 12 revealed primary carcinoma of the liver, and 166, cancers of all types. This indicates an incidence of 0.66 per cent for primary hepatic carcinoma and of 7.2 per cent for cancers of all types in the necropsies at this hospital. In each grouping the ratio is higher than most of the ratios previously reported from this country and Europe. The data on these 12 cases are tabulated. The autopsies revealed no other primary cancer.

Cases of Carcinoma of the Liver Encountered in 1,817 Consecutive Autopsies, 1930 to 1945

Autopsy No.	Type of Tumor	Distribution of Nodules	Necrosis	Hemorrhage	Cirrhosis	Weight	Extrahepatic Metastases	Age	Sex
195	Liver cell	All lobes	Marked	Moderate	Marked	Not recorded	Omentum	35	M
222	Liver cell	All lobes	Slight	Slight	Marked	2,525 Gm.	None	46	M
411	Liver cell	All lobes	Marked	Marked	Slight	4,650 Gm.	Lung	16	M
467	Liver cell	All lobes	Marked	Slight	Moderate	Small	Upper abdominal lymph nodes	68	M
608	Liver cell	All lobes	Marked	Slight	Marked, portal	Greatly enlarged	None	45	M
739	Liver cell	Right lobe	Marked	Slight	Marked	1,300 Gm.	Gastrohepatic nodes and pelvic peritoneum	50	M
1116	Liver cell	All lobes	Slight	Slight	Marked, portal	3,575	None	32	F
1241	Bile duct	All lobes	Moderate	Moderate	Marked	Enlarged	Hilar lymph nodes	58	M
1333	Liver cell	Left lobe	Marked	Marked	Marked	6,340 Gm.	Peritoneum	51	M
1804	Liver cell	All lobes	Marked	Marked	Slight	4,900 Gm.	Lung	67	M
1654	Liver cell	All lobes	Marked	Slight	Moderate	6,900 Gm.	None	49	M
1817	Liver cell	All lobes	Slight	None	Marked	2,100 Gm.	None	52	F

All investigators have agreed on the comparative rarity of primary carcinoma of the liver. Charache [1] in 1939 published a compilation of all cases reported up to August 1937. In 159,762 autopsies he found 808 cases of primary hepatic carcinoma, a percentage of 0.506. These statistics were collected from all parts of the world. The higher ratios were from Oriental and African countries. To these he added 153 other cases in the literature in which the ratio to the total number of autopsies was not given and, also, the 164 cases originally reported by Eggel [2] in 1901, making a total of 1,125 cases. In 1944 Warvi [3] made a comprehensive review of the literature. After eliminating reduplications and adding later cases, including 25 of his own, he concluded that there were 1,200 histologically studied cases.

The geographic and racial distribution of the disease has been noted by many investigators. Gustafson [4] has one of the largest series from a single source. In 24,000 autopsies in Bellevue Hospital, New York, over a period of thirty years the ratio of primary hepatic carcinoma was 0.25 per cent. The incidence among Negroes was parallel to the admission rate of this group but the incidence among Chinese and Japanese was higher than the admission rate.

Earlier reports by Goldzieher and Bokay,[5] of Budapest, Hungary, revealed 18 cases in 6,000 autopsies, a ratio of 0.3 per cent. The same au-

From the Department of Pathology, Howard University Medical School, and Freedmen's Hospital.

1. Charache, H.: Am. J. Surg. **43**:96, 1939.

2. Eggel, H.: Beitr. z. path. Anat. u. z. allg. Path. **30**:506, 1901; cited by Charache.[1]

3. Warvi, W. N.: Arch. Path. **37**:367, 1944.

4. Gustafson, E. G.: Ann. Int. Med. **11**:889, 1938.

5. Goldzieher, M., and Bokay, Z.: Virchows Arch. f. path. Anat. **203**:75, 1911.

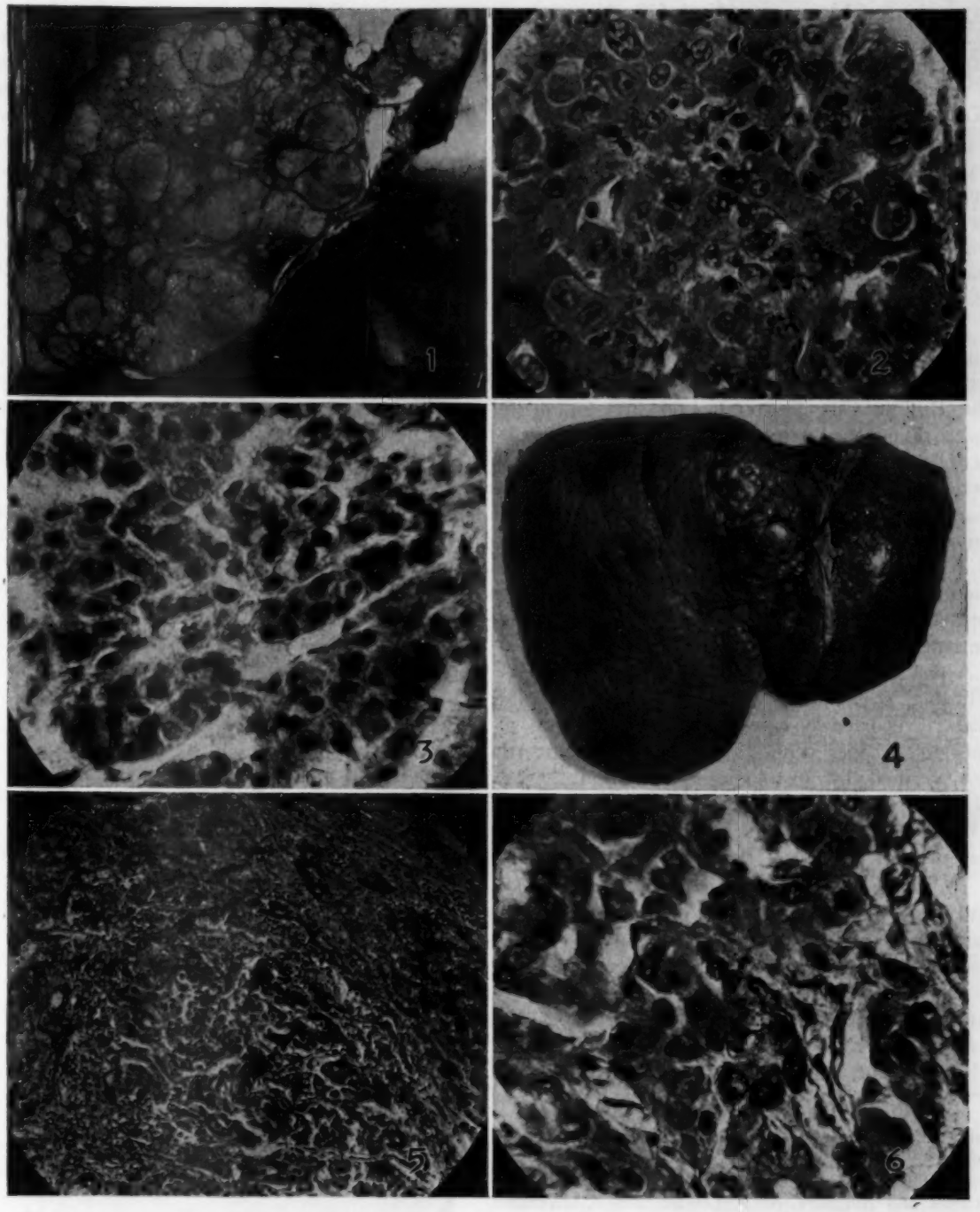

Fig. 1 (autopsy 222).—Gross photograph of the liver showing primary carcinoma (hepatoma) with many nodules of various sizes.

Fig. 2 (autopsy 467).—Photomicrograph showing liver-like structure of neoplastic cells.

Fig. 3 (autopsy 222).—Photomicrograph showing anaplastic cells with hyperchromatic or vesicular nuclei. Some cells have a polygonal shape like that of the liver cell.

Fig. 4 (autopsy 739).—Gross photograph of the liver showing coarse cirrhosis and primary carcinoma (hepatoma).

Fig. 5 (autopsy 739).—Photomicrograph showing liver cells, cirrhosis and well differentiated tumor cells.

Fig. 6 (autopsy 739).—This higher magnification shows the tendency of the tumor cells to maintain hepatic structure, but there are large hyperchromatic nuclei and mitotic figures.

thors cited Orth,[6] who found 2 cancers of this type among 713 cancers (*Krebsfällen*), a rate of 0.28 per cent. From Guy's Hospital, London, Rolleston[7] recorded 24 cases in 18,500 autopsies, a ratio of 0.13 per cent. Among the natives of Kenya Colony, East Africa, Pirie[8] found 36 cases of primary hepatic carcinoma among 91 cases of cancer of all types, a ratio of 39.56 per cent. He attributed the high incidence to the prevalence of schistosomiasis. But in the German colonies of Africa, Hansemann[9] in examination of 200,000 natives found only 21 with cancer, in 4 of whom it was primary in the liver.

At the Mayo Clinic, Counseller and McIndoe[10] found 5 cases in 5,976 autopsies, a ratio of 0.08 per cent. In 3,000 autopsies at the Buffalo City Hospital, Loesch[11] found 14 cases of primary carcinoma of the liver, a ratio of 0.46 per cent. The highest incidence in any series in this country was reported by Hill,[12] of San Antonio, Texas, who found 3 cases in 150 autopsies, a ratio of 2.0 per cent. One of the 3 patients was a 25 year old Negro with a cirrhotic liver. Quinland and Cuff[13] reported primary hepatic carcinoma in 4 of 300 Negro patients with cancers of all types, or a ratio of 1.3 per cent, in Nashville, Tenn. From St. Elizabeth's Hospital, Washington, D. C., Neuman[14] reported 8 cases in 8,200 autopsies, a ratio of 0.1 per cent. Two of his cases were not verified by histologic examination. One of the patients was a Negro woman 55 years old. This is the city in which I found 12 cases in 1,817 autopsies of Negroes.

From the Vancouver General Hospital, Strong and Pitts[15] reported 12 cases of primary hepatic carcinoma in 1,967 autopsies, a ratio of 0.61 per cent. The incidence in white people was 2 cases in 1,828 autopsies, or 0.109 per cent. Among the Chinese he found 10 cases in 139 autopsies, a ratio of 7.19 per cent. All of the Chinese came from the province of Kwantung, in the south of China, where an extremely high percentage of the population is infected with intestinal parasites, chiefly the liver fluke, Clonorchis sinensis. Cirrhosis was present in all of the livers, suggesting previous irritation.

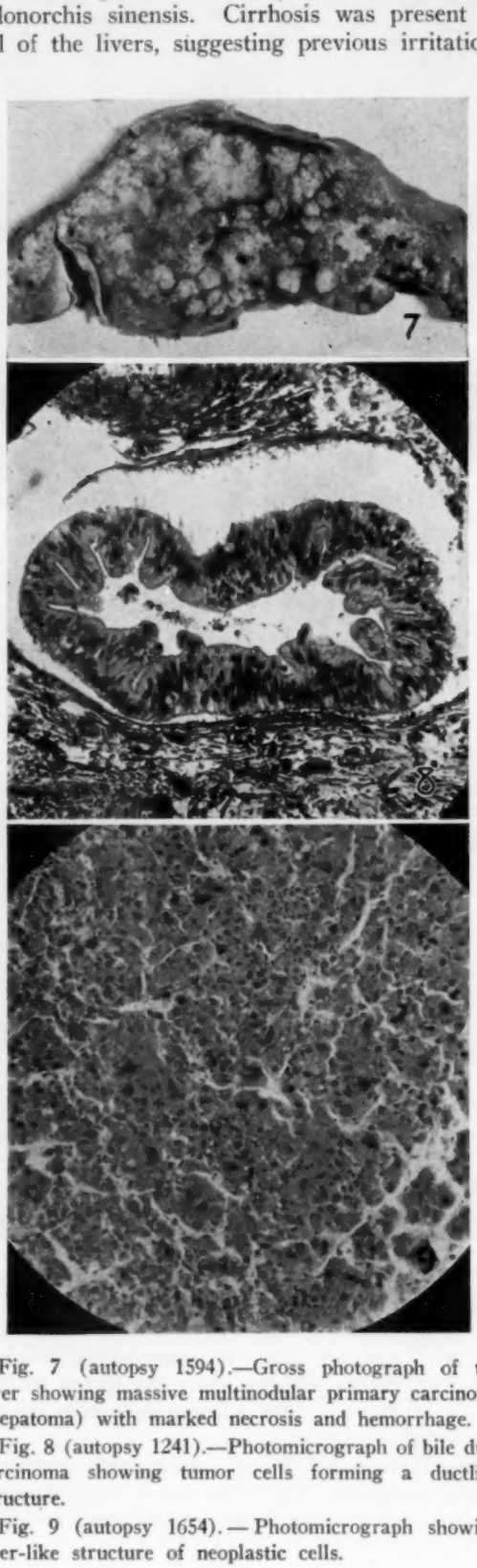

Fig. 7 (autopsy 1594).—Gross photograph of the liver showing massive multinodular primary carcinoma (hepatoma) with marked necrosis and hemorrhage.

Fig. 8 (autopsy 1241).—Photomicrograph of bile duct carcinoma showing tumor cells forming a ductlike structure.

Fig. 9 (autopsy 1654).—Photomicrograph showing liver-like structure of neoplastic cells.

6. Orth, M.: Ueber primäres Lebersarkom, Strassburg, J. H. E. Heitz, 1885; cited by Goldzieher and Bokay.[5]

7. Rolleston, D. R.: Disease of the Liver, Gall Bladder and Bileducts, ed. 2, London, The Macmillan Company, 1912, p. 469.

8. Pirie, J. H.: M. J. South Africa **17**:87, 1921.

9. Hansemann, D.: Ztschr. f. Krebsforsch. **14**:39, 1914; cited by Counseller and McIndoe.[10]

10. Counseller, V. S., and McIndoe, A. H.: Arch. Int. Med. **37**:363, 1926.

11. Loesch, J.: Arch. Path. **28**:233, 1939.

12. Hill, H.: Texas State J. Med. **25**:14, 1929.

13. Quinland, W. S., and Cuff, J. R.: Arch. Path. **30**:393, 1940.

14. Neuman, M. A.: Am. J. Path. **20**:895, 1944.

15. Strong, G. F., and Pitts, H. H.: Ann Int. Med. **6**:485, 1932.

These investigators expressed the belief that the high incidence of hepatic cancer was due to parasitic infection. According to Tull,[16] primary carcinoma of the liver is comparatively frequent among the natives of certain provinces of Southern China, but flukes were not common in Singapore, where his studies were made. All of his cases were among the pauper class.

As to the frequency of primary hepatic cancer in Africans, later reports support those already mentioned. Among the Bantus of South Africa the liver is much the commonest site of primary carcinoma, hepatic cell carcinoma accounting for 37 of 73 epithelial tumors (Strachan[17]). This high incidence was confined to males. The disease occurs at an earlier age than among Europeans. Only 2 of the 37 patients had bilharzial lesions. Vint[18] reported 24 cancers in 1,100 autopsies on natives of Kenya, East Africa, 8 of which were primary carcinoma of the liver; the percentages are 2.18 and 33, respectively. The cancerous livers showed cirrhosis of the hobnailed type. A great majority of the autopsies were made on people under 35 years old.

In its genesis primary hepatic carcinoma has been linked with several lesions, namely: cirrhosis, parasitic infection, syphilis, congenital rests, chronic venous congestion, cholangitis and hepatolithiasis (Sanes and MacCallum[19]). Cirrhosis, according to Ewing[20] and many other authors, must be regarded as both contributory to and coincidental with the tumor growth, occurring in 80 per cent of the cases of hepatoma and 50 per cent of the cases of cholangioma. He expressed the belief that there is little doubt that cirrhosis and the factors that lead to it cause degeneration, followed by regenerative overgrowth, which may become excessive and neoplastic. Cirrhosis, varying from slight to marked, was present in the 12 cases tabulated in this article.

Schistosomiasis has been considered as a factor responsible for the high incidence of the disease among certain Chinese. There was no evidence of parasitic infection in any of the cases listed in the table. Hartz[21] found 5 cases of liver cell carcinoma in 33 autopsies of Chinese men in the Netherland West Indies. In none of these was there gross or microscopic evidence of schistosomiasis. These and other studies led him to agree with Bonne[22] that schistosomiasis does not play a role in the etiology of liver cell carcinoma in the Chinese. It was Bonne[23] who reported the results of an extensive study of carcinoma of the liver among the Malays and the Chinese of the Netherland East Indies. An analysis of 12,957 autopsies revealed primary hepatic cancer in 11 per cent of the Malay men, 3 per cent of the Malay women and 1.4 per cent of the Chinese men. This form of cancer was shown to have the highest incidence in all statistics of autopsies in Netherland East Indies. It was nearly always a liver cell carcinoma developing in a cirrhotic liver and was not due to helminthic infection. He also stated that in Singapore, China, and Manila, P. I., the liver is the organ of highest frequency of primary cancer, while in Tokyo, Japan,[24] the liver ranks second and the figure is much lower. The statistics on Manila are based on a paper by Vedder,[25] who found in autopsies in the Philippine General Hospital that the most frequent site of primary carcinoma was in the liver and the gallbladder. The ratio was 38.7 per cent; he does not give figures on the liver alone.

Experimentally Sasaki and Yoshida[26] produced carcinoma of the liver in mice and rats by adding orthoaminoazotoluene to the diet. The lesions of the liver were multinodular and produced lymphatic and pulmonary metastases. The animals were fed on rice and carrots. If the animals are fed on wheaten cereals, orthoaminoazotoluene will not produce cancer (Boyd[27]).

The protective factors in the diet are casein and riboflavin. Dietary deficiencies, therefore, may explain the racial and geographic distribution of this disease, with social and economic conditions acting as predisposing factors.

SUMMARY AND CONCLUSIONS

A survey of the literature shows that primary carcinoma of the liver is comparatively rare in this country and in Europe. The incidence is much higher among some groups of Orientals and Africans.

16. Tull, J. C.: J. Path. & Bact. **35**:557, 1932.
17. Strachan, A. S.: J. Path. & Bact. **39**:209, 1934.
18. Vint, F. W.: Lancet **2**:628, 1935.
19. Sanes, S., and MacCallum, J. D.: Am. J. Path. **18**:675, 1942.
20. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 738.
21. Hartz, P. H.: Arch. Path. **39**:1, 1945.
22. Bonne, C.: Am. J. Cancer **25**:811, 1935.
23. Bonne, C.: Am. J. Cancer **30**:435, 1937.
24. Nagayo, in Congrès international de lutte scientifique et sociale contre le cancer, 1933, p. 225; cited by Bonne.
25. Vedder, E. B.: J. A. M. A. **88**:1627, 1927.
26. Sasaki, T., and Yoshida, T.: Virchows Arch. f. path. Anat. **295**:175, 1935.
27. Boyd, W.: Text-Book of Pathology, ed. 4, Philadelphia, Lea & Febiger, 1943, p. 536.

To a total of 1,200 cases in the literature 12 cases are added. All the patients were American Negroes. The incidence is seen to be higher in Negroes than in the general population. The data on these additional cases include: age and sex of patient; type of tumor; nodularity; presence or absence of cirrhosis, necrosis, hemorrhage and extrahepatic metastases; weight of the liver when available.

In the genesis of the disease the role played by cirrhosis is important.

Experimental carcinogenesis suggests dietary deficiencies acting as predisposing factors in explanation of the peculiar racial and geographic distribution of the disease.

HÜRTHLE CELL TUMOR OF THE THYROID GLAND IN AN INFANT

WILLIAM J. MORROW, M.D., CHICAGO

An uncommon tumor of the thyroid gland occurring in adults was first described by Langhans [1] in 1907 and has been noted from time to time since. The tumor is known as Hürthle cell tumor. One such growth occurring in an infant was described by Symmers [2] in 1941.

I believe that Hürthle cell tumor in an infant is of sufficient interest and rarity to warrant the report of a case.

REPORT OF A CASE

A 2 month old girl was admitted to the hospital, Nov. 2, 1944, to the service of Dr. Stanley Gibson. She had been referred by Dr. William R. Whitley because of a growing mass in the neck, present since birth but doubling its size in the two weeks prior to admission. Flexion of the neck produced marked respiratory difficulty, and the infant slept with her head extended. A large, lobulated, somewhat soft mass involved the entire anterior portion of the neck in the region of the thyroid gland and extended posterolaterally to the angles of the jaw. Roentgen examination showed the trachea to be surrounded and compressed by the mass. The child was seen by Dr. Albert H. Montgomery and Dr. J. J. Mussil, who agreed that roentgen therapy should be given a trial before resorting to surgery.

Roentgen therapy was started on November 4, and four treatments were given in the next week (each four minutes long, with 144 roentgens), without noticeable change.

Aspiration of the mass with the large bore needle on November 8 revealed some bloody fluid containing large, pale cells with central nuclei. The cytoplasm was vacuolated and contained many small eosinophilic granules. These cells appeared like Hürthle cells.

On November 18, because of severe tracheal compression, the isthmus of the thyroid gland and part of each lateral lobe were removed. The trachea was thereby freed, but the large lateral masses were left intact.

Postoperatively two more roentgen treatments were given. On discharge, November 29, the lateral masses were still large.

The specimen measured 4 by 2 by 1.5 cm. The cut surface was bloody and finely lobular with a yellow-gray color. The capsular surface was smooth and unbroken.

A frozen section showed acini lined by large, pale cells with dark nuclei. A few of the acini showed reticular material, but no typical colloid was seen.

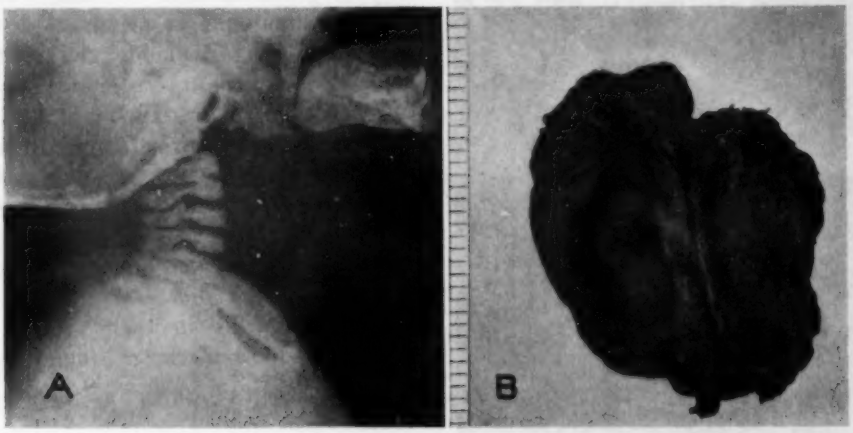

Fig. 1.—*A*, roentgenogram showing the tumor mass surrounding and compressing the trachea. *B*, specimen of Hürthle cell tumor removed at operation. The capsular surface is shown anteriorly. The cut surface is shown above.

Paraffin sections showed a fine connective tissue stroma containing numerous blood vessels, dividing the tissue into lobules. The lobules were composed of irregular follicles lined by polyhedral, cuboid and low columnar cells which were large and pale and contained irregular vacuoles in a pink, finely granular cytoplasm. The nuclei were sometimes eccentric and deep blue. Some of the follicles had invaginations of these cells into the acinous spaces. A few follicles contained small amounts of reddish pink homogeneous material. Scattered throughout the sections were groups of smaller, darker-staining cells with compact cytoplasm. None of the usual thyroid epithelium was seen.

No mitotic figures and no cellular invasion of blood vessels or capsule were seen. A diagnosis of Hürthle cell adenoma of the thyroid gland was made.

The infant returned to the hospital on December 6, one week after discharge, because of diarrhea. This responded to treatment with protein milk and sulfa-

From the Otho S. A. Sprague Memorial Institute and the Children's Memorial Hospital.

1. Langhans, T.: Virchows Arch. f. path. Anat. **189**:69, 1907.

2. Symmers, D.: Arch. Path. **31**:99, 1941.

thiazole. At this time the mass was almost gone. It had "melted away" in seven days.

During this admission the blood phosphorus was 6.9 mg. per hundred cubic centimeters, the phosphatase was 11.5 Bodansky units, and the cholesterol was 183 mg. per hundred cubic centimeters, giving no evidence of faulty thyroid or parathyroid function.

The report three months after the last discharge was that the infant (aged 7 months) was well and the tumor mass gone.

The case presented is comparable to another —Symmers'[2] case of congenital Hürthle cell tumor. This, like Symmers' case, is histologically one of adenoma.

Unlike most of the tumors reported, this one consists entirely of Hürthle cells and is not associated with other elements of the thyroid gland,

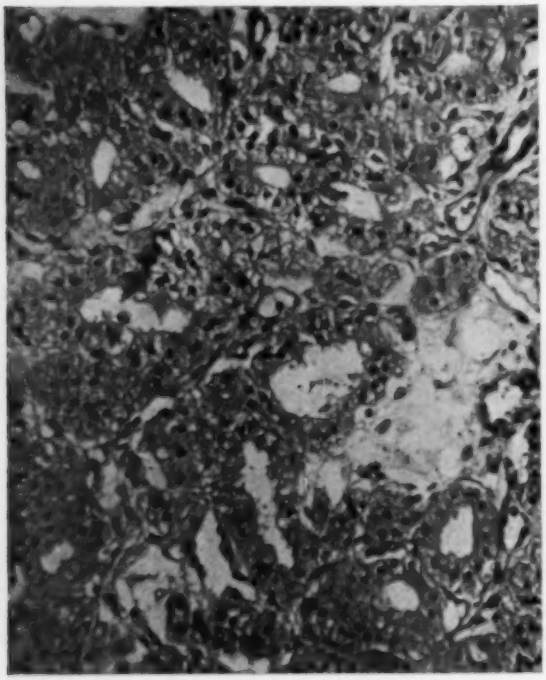

Fig. 2.—Photomicrograph of Hürthle cell tumor (× 400) showing acini, islands and cords of large, pale cells in a vascular connective tissue stroma. Note the variously shaped and sized acini around the central clear area.

either normal or abnormal. The tumor mass examined showed no elements other than Hürthle cells except for a few scattered darker cells, which may have been Hürthle cells changed by roentgen therapy.

Figures 1 and 2 show the tumor surrounding the trachea, the gross specimen and the histologic structure of the tumor.

THE HÜRTHLE CELL

In 1881 Baber[3] described an unusual type of cell in the thyroid gland. It was a large cell with cytoplasm paler than that of the usual acinous cell. Langendorff[4] in 1889 described thyroid cells of like nature. A similar cell was described by Hürthle[5] in 1894, which he thought was an interfollicular cell and one which had the potentiality of developing into normal thyroid epithelium.

Somewhat later Getzowa[6] brought forth the view that Hürthle cells were remnants of the ultimobranchial (postbranchial) bodies or lateral thyroid anlage.

Eisenberg and Wallerstein[7] concluded that the Hürthle cell was identical with the oxyphilic cell of Welch or one of the cell types of the parathyroid gland. These observers stand alone in this view of the origin of the Hürthle cell.

The Hürthle cell is believed by others (Wilensky and Kaufman[8]) to represent a physiopathologic rather than an anatomic variant of the thyroid cells. They believe that Hürthle cells may arise from normal or abnormal cells in the thyroid gland because of changes in the gland, such as nutritional or metabolic disturbances.

Mueller[9] and Michaud[10] did not find Hürthle cells. Boéchat[11] in his early study did not mention the cell. Maximow and Bloom[12] after careful studies of normal thyroid glands decided that Hürthle cells were dead or dying cells or epithelial cells cut tangentially. Horsley[13] found embryonal thyroid cells below the thyroid capsule definitely encapsulated and not within the gland. Wilson[14] and Williamson and Pearse[15] found no Hürthle cells in their studies. However, Nonidez[16] and Zechel[17] found parafollicular

3. Baber, E. C.: Phil. Tr. Roy. Soc., London **172**:577, 1881.
4. Langendorff, O.: Arch. f. Anat. u. Physiol., 1889, supp., p. 219.
5. Hürthle, K.: Arch. f. d. ges. Physiol. **56**:1, 1894.
6. Getzowa, S.: Virchows Arch. f. path. Anat. **188**:181, 1907.
7. Eisenberg, A. A., and Wallerstein, H.: Arch. Path. **13**:716, 1932.
8. Wilensky, A. O., and Kaufman, P. A.: Surg., Gynec. & Obst. **66**:1, 1938.
9. Mueller, L. R.: Beitr. z. path. Anat. u. z. allg. Path. **19**:127, 1896.
10. Michaud, L.: Virchows Arch. f. path. Anat. **191**: 63, 1908.
11. Boéchat, P. A.: Recherches sur la structure normale du corps thyroïde, Thesis, Paris, no. 155, 1873, p. 12.
12. Maximow, A. A., and Bloom, W.: Textbook of Histology, ed. 2, Philadelphia, W. B. Saunders Company, 1934, p. 305.
13. Horsley, V.: Lancet **2**:1163, 1886.
14. Wilson, G.: Anat. Rec. **37**:31, 1927.
15. Williamson, G. S., and Pearse, I. H.: J. Path. & Bact. **26**:459, 1923.
16. Nonidez, J. F.: Am. J. Anat. **49**:479, 1931-1932.
17. Zechel, G.: Surg., Gynec. & Obst. **52**:228, 1931; **54**:1, 1932.

Author	Case	Sex	Age	Signs and Symptoms	Adenoma or Carcinoma	Comment
Langhans [1]	1	F	55	Tumor rapidly growing for 3 mo.	?	
	2	M	68	Rapid growth of goiter for 4 weeks with respiratory difficulty	Metastases to lungs and liver; death from tumor; carcinoma	
	3	F	41	Thyroidectomy 12 yr. before large tumor; recurrence with rapid growth for 9 mo.	Metastases to lungs; death from tumor; carcinoma	
	4	F	41	Rapidly growing tumor	?	
	5	M	32	Large tumor—one mass lemon sized, the other egg sized	?	
Lobenhoffer, W.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **20**: 650, 1909	6	F	12	Rapidly growing goiter with symptoms of tracheal pressure	Considered as adenoma	
	7	F	40	Goiter	Considered as adenoma	
Haagensen [23]	8	F	37	1918 enlarged thyroid gland with menorrhagia and nervousness; 1924 partial thyroidectomy	Tumor invaded capsule; grade 2 small alveolar, large cell carcinoma	Tumor slowly decreased in size with roentgen therapy
	9	F	58	1906 goiter grew slowly; 1915 tumor removed; 1926 cough, hemoptysis; hard, irregular mass at the base of the neck; 1929 recurrence of cough and increase in size of neck; back pain	1925 tumor recurred and was treated with radon; 1926 pulmonary metastases by roentgenogram; 1929 metastases to spine by roentgenogram; grade 2 Hürthle cell carcinoma	1926 roentgen therapy with improvement; 1931 neck and chest unchanged; vertebrae more involved
Eisenberg and Wallerstein [7]	10	F	54	1930 small goiter, larger in past 5 mo.; spell of fainting and muscular twitching; basal metabolic rate (B.M.R.) +40 reduced to +18 with strong solution of iodine U. S.P.; rt. lobe removed—"toxic nonexophthalmic goiter"; 1931 B.M.R. +41; lt. lobe removed	Hürthle cell adenoma	Muscular twitchings used as evidence of hyperparathyroidism although increased muscular irritability would be more likely in hypoparathyroidism
Eberts [25]	11	F	43	Enlargement of neck, nervousness, palpitation, loss of weight; heat intolerance; B.M.R. +68; pulse 120; tumor removed; B.M.R. +1 and pulse 70 18 days postoperatively	Tumor encapsulated; areas of Hürthle cells demarcated from parent adenomatous tissue but not encapsulated; no mitotic figures seen and no invasion of blood vessels; considered as carcinoma	Hürthle cells present with thyroid adenoma
Wilensky and Kaufman [8]	12	F	47	4½ yr. of arthralgia, 6 mo. of palpitation, tremor, sweating; weight loss of 40 lb. (18 Kg.); B.M.R. +2	Adenomatous growth of Hürthle cells in lymphocytic stroma with areas of early carcinoma also seen; no recurrence in 3 yr.; adenoma	Hürthle cells mixed with adenoma and early carcinoma
	13	F	47	2 yr. of increased swelling of neck nervousness, sweating; B.M.R. +22	Cords of Hürthle cells; no recurrence in 8 mo.; considered as adenoma	
Martin and Elkin [24]	14	F	46	10 yr. of enlargement of neck; 2 yr. prior to examination patient said to have thyrotoxicosis; B.M.R. normal	Numerous islands of compact Hürthle cells forming acini; adenoma	
	15	F	38	5 yr. of enlargement of the thyroid gland; dysphagia; trachea displaced to left; nodule of rt. lobe; B.M.R. +22	Hürthle cells between many cystic spaces; adenoma	
	16	F	54	2 small nodules in neck growing larger; hoarseness; 25 lb. (11 Kg.) weight loss in 2 yr.; numerous nodules adjacent to thyroid gland	Large Hürthle cells attempting to form acini; many mitotic figures; Hürthle cell carcinoma	
Symmers [2]	17	F	6 weeks	Large swelling of thyroid gland since birth, growing larger; respiratory distress necessitating emergency operation, in which two large masses were removed	Lobules of alveolar formation of large, pale cells with small, rather dense nuclei (Hürthle cells); patient discharged improved in 3 mo.; congenital Hürthle cell tumor, histologically adenoma	Tumor was present from birth
Harry [22]	18	M	48	4 yr. previously well encapsulated Hürthle cell tumor removed; tumor recurred widely	Two subsequent operations to remove widely recurring tumor infiltrating neck; carcinoma	

Reported Cases of Hürthle Cell Tumor—Continued

Author	Case	Sex	Age	Signs and Symptoms	Adenoma or Carcinoma	Comment
Reiman, D. L.: Bull. School Med. Univ. Maryland **28**:93, 1943	19	F	49	Lump in neck for 2 yr.; growing slowly; 20 to 30 lb. (9 to 13.5 Kg.) weight loss; patient irritable, nervous; heat intolerance, with B.M.R. +11, +19	Cords, columns and sheets of Hürthle cells; histologic appearance adenoma	Calcium 10.3 mg. and phosphorus 4.7 mg. per 100 cc. (normal)
Morrow, this issue....	20	F	2 mo.	Large thyroid since birth, growing double in size in the 2 weeks prior to admission; operation necessary because of tracheal compression	Tumor composed of Hürthle cells in lobules of irregular follicles; no invasion of capsule or of blood vessels; no mitotic figures; tumor gone 3 mo. after discharge; histologic appearance adenoma	Tumor, present from birth, disappeared following roentgen therapy; normal phosphorus (6.9 mg. per 100 cc.), phosphatase (11.5 Bodansky units), and cholesterol (183 mg. per 100 cc.)

Summary

Total Cases	Sex Distribution			Age Distribution					Diagnosis			Cases in Which Hyperthyroidism Was Associated
	Female	Male	?	40 Years and Over	20-40 Years	10-20 Years	Under 1 Year	?	Adenoma	Carcinoma	?	
23	17	3	3	14	3	1	2	3	10	8	5	9

cells (Hürthle cells) in dogs, while Raymond [18] found them in rabbits.

Differing views exist therefore as to the occurrence and the origin of the Hürthle cell.

HÜRTHLE CELL TUMOR

Tumors composed of Hürthle cells and variously described as Hürthle cell tumor, Baber cell tumor, Langhans tumor, Getzowa struma, struma postbranchialis, Wurchende struma, and small alveolar, large cell carcinoma of the thyroid gland have been described since 1907.

Up to the present thirteen different reports totaling 23 cases of Hürthle cell tumor have been made (table). This includes the present report.

In addition to those shown in the table 3 other cases of Hürthle cell tumor have been reported. In the third edition of "Neoplastic Diseases" Ewing [19] mentioned having examined 2 tumors of this type but gave no clinical data and made no definite statement as to their nature, adenomatous or carcinomatous. Clute and Warren [20] reported 1 case of Hürthle cell tumor among 226 cases of thyroid cancer, which they considered to be a case of adenocarcinoma. Wegelin [21] mentioned having seen tumors of the Hürthle cell type at all ages but gave no data. He considered all of them as adenoma.

The data show that Hürthle cell tumor occurs in females much oftener than in males (17 females, 3 males). It is usually found in adults over 40 (14 cases). Only 3 cases of Hürthle cell adenoma of a child have been reported. One patient was a 12 year old girl; the other 2 were infants.

Ten of the cases listed were grouped as cases of adenoma; 8, as cases of carcinoma. In 5 cases the tumor type was not reported or was unknown. Hyperthyroidism was associated with the tumor in 9 cases.

The treatment and the prognosis in cases of Hürthle cell tumor vary as much as do the ideas as to its origin and nature.

Those who consider the tumor cancerous advocate surgical eradication and roentgen or radium therapy (Harry [22]; Haagensen [23]; Wilensky and Kaufman [8]). Martin and Elkin [24] have advised early surgical removal in that it is frequently impossible to recognize carcinoma until metastases occur.

The tumor, if it is carcinomatous, appears to grow fairly slowly, and in all of the recent cases the patient was alive at the time of recording. One of Haagensen's [23] 2 patients was alive sixteen years after the original tumor was removed; the other twelve years after the tumor was first noticed. Ebert's [25] patient had had a thyroid mass for four years. Wilensky and Kaufman [8] reported 1 case in which a thyroid mass was present for three years and Hürthle cell tumor and thyroid carcinoma were distinguished in the same gland. In 1 of the 3 cases observed by Martin and Elkin,[24] a cancerous tumor presumably had been present for three years. In Harry's [22] 1 case recurrences necessitated reoperation twice, but the patient was alive four years after the first tumor was removed.

18. Raymond, N.: Anat. Rec. **53**:355, 1932.
19. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928, pp. 952-953.
20. Clute, H. M., and Warren, S.: Surg., Gynec. & Obst. **60**:861, 1935.
21. Wegelin, C., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, p. 199.
22. Harry, N. M.: Roy. Melbourne Hosp. Clin. Rep. **12**:18, 1941.
23. Haagensen, C. D.: (supp.) Am. J. Cancer **15**: 2063, 1931.
24. Martin, J. D., Jr., and Elkin, S. C.: Ann. Surg. **110**:169, 1939.
25. Eberts, E. M.: Canad. M. A. J. **28**:650, 1933.

Wilensky and Kaufman [8] pointed out that the prognosis depends on the type of cell from which the tumor arises.

From the available information it appears that Hürthle cell tumor may be either adenoma or carcinoma, but for ultimate safety it may be best to consider it potentially as carcinoma and treat it as such.

In the present state of knowledge it may be said that the Hürthle cell tumor is a growth in, or in the region of, the thyroid gland which consists of large, pale cells having finely granular eosinophilic vacuolated cytoplasm and variously placed nuclei. These cells may form cords, islands or acini. The cells may appear as part of an adenomatous mass in association with normal thyroid tissue, "hyperthyroid" tissue, thyroid carcinoma or as a tumor of uniform structure.

The variations mentioned make the theory of Wilensky and Kaufman [8] that the Hürthle cell is not a distinct cell but a physiopathologic variant of some cell in the thyroid gland acceptable because it fits all cases and is less specific than other ideas of origin of the cells. Acceptance of the theory would explain why in some instances the tumor is adenoma and in others carcinoma.

The occurrence of "congenital" tumors of this type in 2 young infants may be best explained as an overgrowth of embryonal thyroid cells.

SUMMARY

The origin of the Hürthle cell is in doubt. It may be an embryonal interfollicular cell, a remnant of the lateral thyroid anlage, an ectopic parathyroid cell or a physiopathologic variant of some type of thyroid cell.

Twenty-three cases of Hürthle cell tumor have been reported. The tumor occurs most commonly in females over the age of 40 years It is associated in some cases with signs and symptoms of hyperthyroidism. In 10 cases it was described as adenoma; in 8, as carcinoma. In 5 the tumor type was not stated or was unknown. Prognosis is difficult and depends on what the tumor shows microscopically and clinically. If the tumor recurs, it usually is fairly slow growing.

When the tumor has occurred in children and infants (3 cases) it has been regarded as adenomatous, but definite conclusions are not yet available.

A second case of so-called congenital Hürthle cell adenoma in an infant has been observed.

CARCINOMA OF THE STOMACH WITH MULTIPLE ANNULAR METASTATIC INTESTINAL INFILTRATIONS

ELWYN L. HELLER, M.D., PITTSBURGH

Carcinoma of the stomach, being one of the most common of cancers and most diversified as to anatomic and histologic types, frequently presents unusual features incident to its variability. Some of its peculiarities were exemplified in the case to be reported, the diagnosis of which was dependent on microscopic study.

REPORT OF A CASE

A 72 year old white woman was admitted to the Presbyterian Hospital on Jan. 8, 1945, complaining of intermittent colicky abdominal pain of four months' duration, recently accompanied by diarrhea, loss of weight and vomiting. A few days before admission all bowel movements ceased.

She had been treated for hypertension for many years and was recently digitalized for signs of congestive heart failure. An annular stenosing mass was noted in the rectal wall, interpreted as carcinoma, for which a first stage colostomy was performed. Following the operation, signs of rapidly progressing congestive heart failure developed, and she died on the first postoperative day.

Autopsy.—The postmortem examination was performed two hours after death. The heart was enlarged and of hypertensive type. There were bilateral hydrothorax, passive congestion of the lungs, the liver and the spleen, and 700 cc. of ascitic fluid in the peritoneal cavity. The ascitic fluid was clear.

In the superior mediastinum was a lemon-sized cyst of milky color, the wall of which was paper thin. On section the cyst collapsed, with the escape of chylous fluid. The inner lining was smooth and glistening. Thymic remnants could be identified in the superior portion of the wall.

The peribronchial lymph nodes were not significantly enlarged.

The stomach externally was normal in size and appearance after separation of fibrous adhesions which bound its serosa to the omentum and to loops of small intestine. The mucosa was diffusely hyperemic and indurated, boggy, thickened and coated with tenacious mucus. The rugae were maintained. In the cardia near the esophageal orifice the mucosa showed an indistinct plaquelike elevation 3 cm. in diameter with slight central depression but no ulceration (fig. 1). Except for its slight elevation (3 mm.) it was not unlike the adjacent mucosa in appearance. The submucosa and the muscularis appeared diffusely thickened, pale and indurated, most marked throughout the fundus.

Scattered irregularly throughout all portions of the small intestine were short annular constricted segments covered by thickened, irregularly opaque, smooth hyperemic serosa (fig. 2). Four such well developed lesions were noted along the small intestine, one of which appeared almost totally obstructive. The intestinal wall in these areas was thickened, pale, fibrous and indurated. Similar but less developed infiltrations could be detected on close scrutiny at several other areas. The mucosa of the involved segments was mamillated and hyperemic but not ulcerated. Similar lesions were noted in the transverse colon and the rectum. None of the mesenteric or retroperitoneal lymph nodes were notably enlarged. The thoracic duct was not examined. Careful examination of all other viscera disclosed no trace of metastatic lesions.

Microscopic Examination.—All sections of the stomach revealed a diffusely invasive tumor process, for the most part submucosal but identifiable in all layers. The cells were discrete, large, oval or polygonal, with slightly acidophilic cytoplasm, often containing secretory droplets that at times displaced the nucleus to the periphery of the cell in signet ring fashion. The nuclei generally were vesicular and round or oval but were flattened or crescentic when adjacent to the cytoplasmic droplets. Mitotic figures were infrequent. At the site of the mucosal plaque in the cardia (fig. 3), the tumor completely replaced the normal mucosa. A striking feature was the obvious spread through intramural lymphatic channels and interstices. No attempt at gland formation could be detected, and for the most part the cells were discrete without cytoplasmic continuity except where crowded within the larger lymphatic vessels.

Sections through the annular lesions of the small intestine and colon revealed diffusely infiltrating tumor, generally within the lymphatic channels, throughout all layers of the intestinal wall. The connective tissue stroma showed signs of proliferation but was not typically scirrhous in character. The tumor cells were similar to those of the primary gastric lesion except for lessened production of cytoplasmic secretory globules. On casual observation they resembled large mononuclear phagocytes (fig. 4).

In the superior portion of the mediastinal cyst small lobules of thymic tissue were surrounded by large, irregular sinusoidal spaces distended with tumor cells and scattered inflammatory cells (fig. 5). The cyst was composed of a thin outer layer of collagenous connective tissue lined with endothelium.

In the peribronchial lymph nodes tumor cells were freely distributed throughout the sinusoidal spaces, but the involvement was considerably less than that of the thymic area.

Additional sections revealed a light diffuse tumor cell infiltration of the hepatic capsule, the adventitial lymphatic channels of the splenic vein (pancreas section), the peripelvic renal tissue, the periadrenal tissue, the omentum and the left fallopian tube. In all sections the involvement was predominantly of lymphatic vessels, with minimal extension to peritoneal surfaces.

Sections from the liver, the lungs, the ovary and other viscera were free from metastatic lesions.

From the Department of Pathology, University of Pittsburgh, and the Presbyterian Hospital.

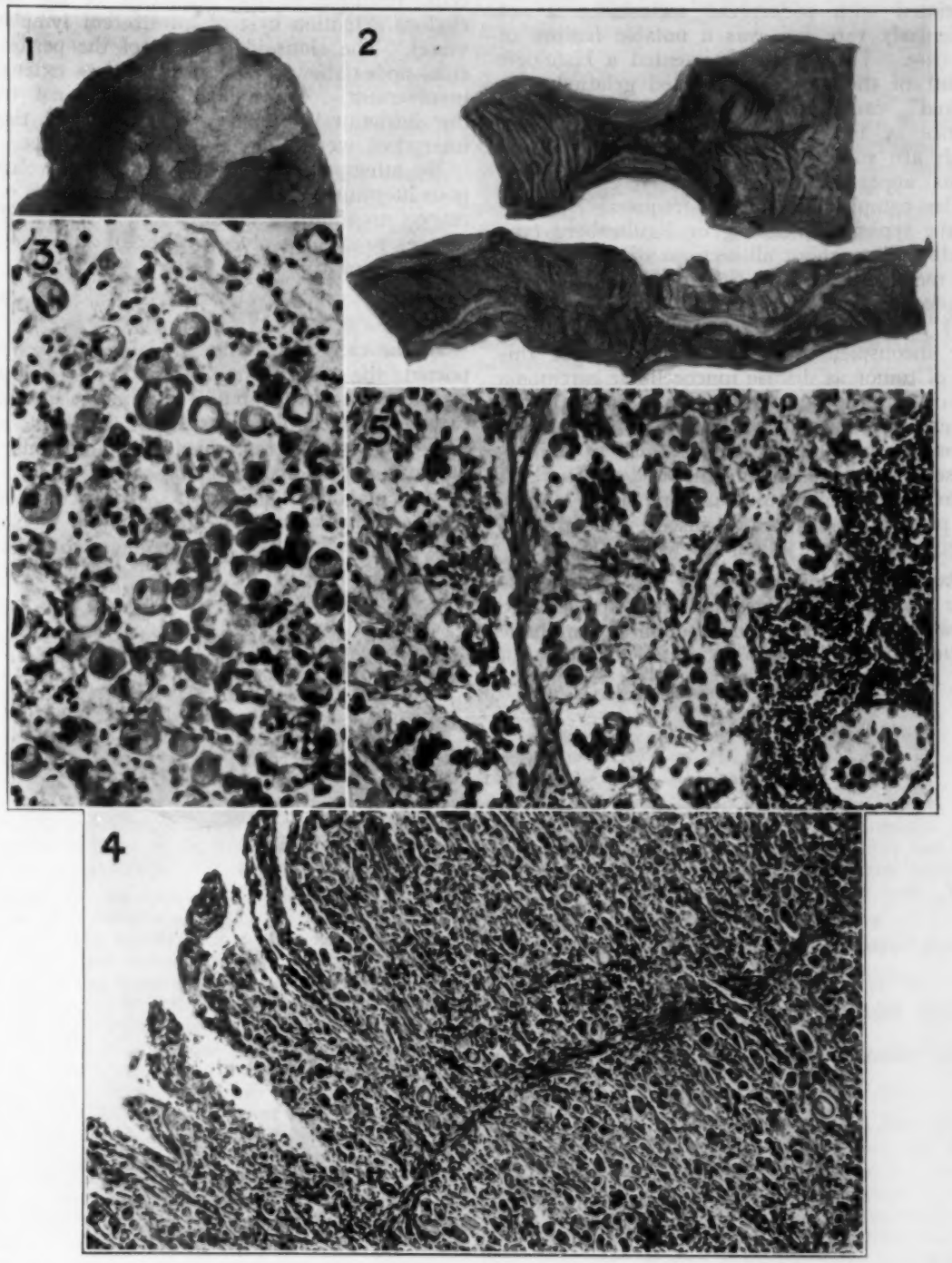

Fig. 1.—Cardiac end of the inverted stomach. The poorly defined mucosal plaque lies in the upper central position.

Fig. 2.—Annular stenosing carcinomatous infiltrations of the small intestine.

Fig. 3.—Diffuse mucocellular carcinoma of the stomach, primary lesion. × 290.

Fig. 4.—Secondary carcinomatous infiltration of the small intestine. The mucosal epithelium has been superficially eroded. × 115.

Fig. 5.—Diffuse carcinomatous infiltration of thymic lymphatic vessels. × 155.

COMMENT

A relatively small and somewhat obscure primary growth occurring in the stomach and associated with widespread metastases is not particularly rare but was a notable feature of this case. The tumor represented a histologic variant of the mucous, so-called gelatinous or "colloid" carcinoma, exhibiting almost total absence of the interstitial deposits of mucus which are so characteristic of the type. The mucus appeared almost exclusively as intracellular cytoplasmic droplets frequently producing the typical signet ring or Krukenberg type of cell. Throughout all sections of the primary and metastatic lesions the tumor exhibited a remarkable fidelity of pattern, in which syncytial masses and even cytoplasmic continuity were inconspicuous. The designation of this type of tumor as diffuse mucocellular carcinoma appears appropriate. The most unusual and interesting feature of the case was the annular stenosing carcinomatous infiltrations of numerous segments of small intestine and colon which resembled granulomatous inflammatory lesions. The picture was further confused by the absence of gross metastatic lesions elsewhere.

The peculiar localization of the gross metastatic lesions to the intestinal tract appeared to be the result of retrograde abdominal lymphatic dissemination following an initial blockage of the mediastinal lymphatic channels. The interstitial lymphatic spaces of thymic remnant were distended and presumably blocked by tumor cells, resulting in the formation of the large chylous retention cyst of an efferent lymphatic vessel. The sinusoidal spaces of the peribronchial nodes showed a similar but less extensive involvement. Throughout the intestinal tract the intramural lymphatic channels and tissue interstices were plugged with tumor cells.

No attempt was made to review the voluminous literature on carcinoma of the stomach with respect to multiple intestinal metastases. References to such involvement appear infrequently in the literature.[1]

SUMMARY

In the case of carcinoma of the stomach reported, the somewhat obscure primary lesion was associated with multiple annular stenosing carcinomatous infiltrations of the small intestine and colon, which resembled granulomatous inflammatory lesions. No other secondary involvement was in evidence. The peculiar distribution was apparently the result of blockage of mediastinal lymphatic channels and retrograde dissemination of the tumor cells through the abdominal lymphatic vessels.

1. Ewing, J,: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 706.

INTRAUTERINE INJECTION OF LYSOL AS AN ABORTIFACIENT

REPORT OF A FATAL CASE COMPLICATED BY OIL EMBOLISM AND LYSOL POISONING

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The disinfectant Lysol is a mixture of cresols (50 per cent) in saponified linseed oil. It is a yellowish or brownish liquid of syrupy consistency. It is translucent, mixes easily with water and gives a strong alkaline reaction on red litmus paper. When applied to the human body, it acts as a powerful local corrosive on account of its cresol and alkaline components. After absorption it produces definite toxic changes in the internal organs, which may be sufficient to cause death. In the case reported here the drug was injected into the uterus for the purpose of inducing abortion, but before that aim was accomplished, the patient died of a combination of oil embolism and the poisonous action of the solution itself.

REPORT OF CASE

A white woman of 26 years was admitted to a hospital with the history of a sudden collapse following a coughing spell during which she brought up much blood-stained fluid from the air passages. She was semicomatose and in severe shock, with rapid, labored respirations, a weak, rapid, thready pulse, blood pressure so low that it could not be recorded, and a cold and clammy skin. There was no obvious cyanosis. Considerable amounts of blood-stained fluid were being coughed up, and the lungs were filled with noisy, moist rales. She died in coma seventy-five minutes after her admission. The only medication which she received was theophylline and ethylenediamine metrazol, and 1,000 cc. of dextrose solution by intravenous injection—all intended to combat shock.

Necropsy (sixteen hours post mortem).—The body was that of a young white woman, 153 cm. in length, 67 Kg. in weight, heavily built and well nourished. The face and neck had a dusky cyanotic hue.

The uterus was enlarged and globular in shape, 25 by 18 by 15 cm., and was distended by an intact amniotic sac containing slightly cloudy straw-colored fluid in which floated a male fetus of four and a half months' gestation. The placenta was attached normally to the anterior surface of the fundus, and there were no signs of separation. The cervix uteri was relaxed, and there was a small amount of blood and blood clot at the outlet; there were not any visible tenacular marks on the external os. About the midportion of the anterior surface of the uterus, the amniotic sac was separated from the uterine wall over an area 10 cm. in diameter, and in this space was 15 cc. of light yellowish brown creamy exudate from which exuded an odor like that of Lysol. At this point the uterine wall was 10 to 15 mm. in thickness and was soft and spongy, and the venous sinuses were distended and ovoid in shape; some near the peritoneal surface measured 5 by 3 mm. and contained a coherent brown-red blood clot. The uterine tissues over this area were flabby and were stained a dull light brownish red hue as though colored by hemolyzed blood. The tubes and ovaries were natural; a corpus luteum was present in the left ovary.

The heart weighed 230 Gm. The right ventricle was distended. The cavities contained considerable fluid blood and a little yellow postmortem clot. There was no foramen ovale. The aortic intima was stained a dull dark red from hemolyzed blood.

The right lung weighed 650 Gm. and the left 550 Gm. Both lungs were free in the thoracic cavity, were well aerated and had a dusky cyanotic hue. The pleural surfaces presented a marbled appearance with small bluish gray irregular areas of edema interspersed between yellowish gray aerated areas of similar size and shape. On section, the alveolar walls were thickened, and areas of consolidation, 2 to 4 mm. in diameter, were present here and there. The air passages contained foamy blood-stained fluid.

The kidneys together weighed 320 Gm. The cortex was thickened and had a dull yellowish gray color. The surface of the kidney presented minute reddish hemorrhagic areas, which were visible only under a magnifying glass. The bladder contained about 300 cc. of a clear straw-colored urine.

The stomach was normal but contained 500 cc. of a dark brown-red, blood-stained fluid in which was considerable mucus.

The brain was congested but otherwise revealed no abnormality. The liver and the spleen were normal. The other organs were normal.

Toxicologic Examination.—The materials saved for analysis included brain, liver, stomach contents and urine, which during the process of collection had become contaminated with some of the exudate between the amniotic sac and the anterior uterine wall. The report was as follows:

"Contaminated urine: alkaline to litmus paper; phenol group present.
"Liver: phenol group present.
"Brain: phenol group present; ethyl alcohol present, 0.25 per cent.
"Stomach contents: phenol group not present; ethyl alcohol present in small amount.
"Other poisons absent."

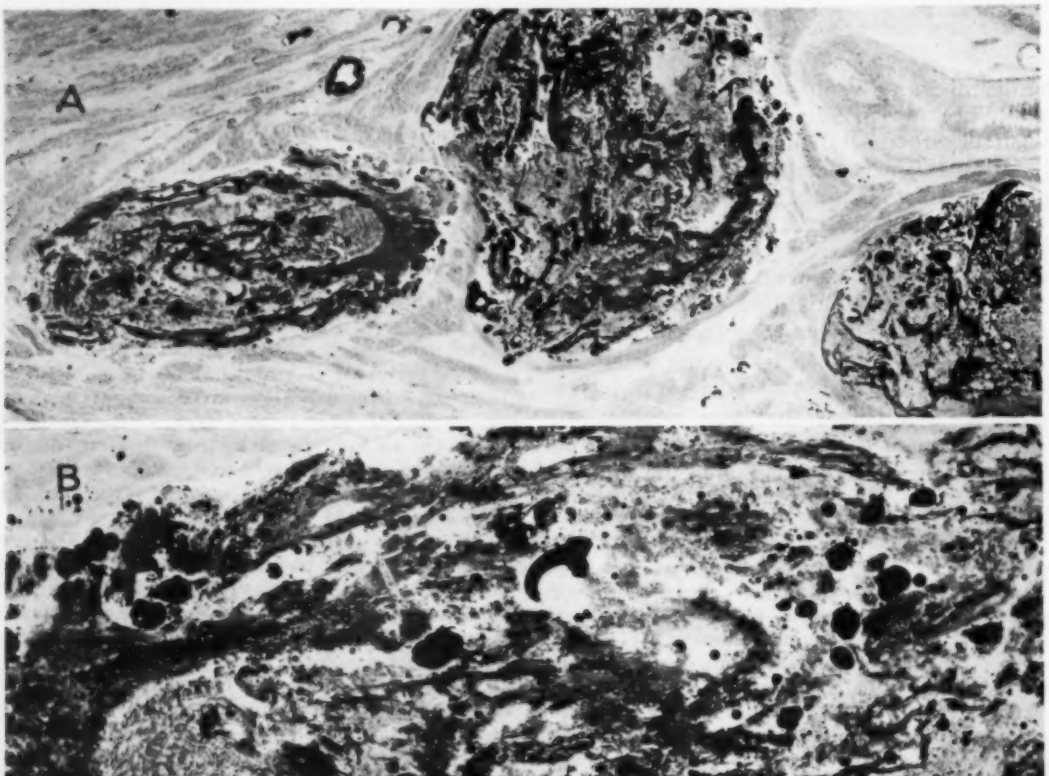
Microscopic Examination.—Sections were made of the lungs, the myocardium, the liver, the spleen, the kidneys, the pons varolii and basilar artery, and the uterine wall and stained with hematoxylin and eosin. Other sections of the lungs, the uterine wall, the myocardium, the kidneys and the pons were stained with Marchi fluid. Still other sections of the lungs, the uterine wall and the kidneys were stained with a potassium ferrocyanide solution and acid alcohol, but iron-containing pigment was not demonstrated.

(*a*) Anterior Wall of the Uterus Near the Exudate: The venous sinuses near the inner lining were filled with granular red-brown blood pigment or shrunken dark-staining red blood cells. The large venous sinuses near the peritoneal layer contained irregular fragments

From the Office of the Chief Medical Examiner, City of New York.

of dark reddish brown blood pigment permeated with circular holes of varying size, while some of the blood pigment was finely granular and was permeated by the same circular holes. The Marchi stain disclosed that all the circular spaces represented oil globules, and these reaction in the uterine tissue, but in places numerous polymorphonuclear leukocytes occurred in some of the smaller arteries.

(*b*) Lungs: The alveolar walls were thickened, and the capillaries contained numerous polymorphonuclear



A, section of uterine wall showing oil globules in venous sinuses; × 5. *B*, large uterine venous sinus containing chemically altered blood and imprisoned oil globules; × 14. *C*, section of lung showing severe oil embolism; × 95.5.

were present in most of the venous sinuses, both large and small; the smaller globules were circular in shape, while the larger ones had an irregular ring form. (See figure, *A* and *B*.) There was only a little inflammatory leukocytes. The Marchi stain demonstrated the presence of oil emboli in such numbers that the pattern of the embolism outlined the alveolar wall (*C* in figure). Some of the emboli were globular in form and were arranged

like a string of beads; others formed a yeast cell pattern; others had long or short vermicular shapes; a few larger emboli filled some of the smaller arterioles. Red blood cells were present in some of the blood vessels, but most of the blood was hemolyzed or laked. A few of the smaller arteries and arterioles contained numerous polymorphonuclear leukocytes and occasionally fragments of dark reddish brown blood pigment similar to the pigment in the uterine venous sinuses. The alveoli in some areas were filled with fluid, and a few alveolar groups showed an exudate of polymorphonuclear leukocytes and large cells of the macrophage type whose cytoplasm was dotted with black pigment granules. The bronchi were normal except for moderate leukocytic infiltration in the wall.

(*c*) Kidneys: Some polymorphonuclear leukocyes were present in the glomerular coils. Many capsules of Bowman contained granular albuminous fluid. The tubular epithelium was not swollen, but many of the tubules in both the cortex and the medulla were filled with a granular albuminous content or with dark reddish brown globules of uneven size, slightly smaller than erythrocytes. The vessels, especially those in the pyramids, were full of blood pigment, laked blood or polymorphonuclear leukocytes. The Marchi stain disclosed a few oil emboli in many of the glomeruli. The interstitial tissue was normal.

(*d*) Brain: A few oil emboli were present in the smaller arteries, but lesions were not seen in the brain tissue. The blood in the blood vessels was mostly hemolyzed.

(*e*) Heart Muscle: A few oil emboli were present in the capillaries of the heart muscle, but the adjacent fibers were unchanged. The blood vessels contained laked blood.

(*f*) Liver: The liver cells were slightly swollen but did not contain visible fat. Numerous polymorphonuclear leukocytes, some of which were eosinophils, were present in the sinusoids. The blood in the veins was laked.

(*g*) Spleen: The sinusoids contained polymorphonuclear leukocytes, round cells, a few eosinophils and macrophages. Brownish pigment dust was present in the blood vessels and the splenic parenchyma. The follicles were normal.

EXPERIMENTAL STUDY AND POLICE INVESTIGATION

An experiment was tried in vitro to determine the behavior of Lysol when mixed with organic fluid material. One cubic centimeter of Lysol was diluted with about the same amount of distilled water, and to this was added a centimeter of human blood. The mixture was transformed immediately into a light yellow-brown, creamy material, which was examined microscopically as a wet preparation. On the slide, oil globules began to separate out in a couple of minutes, and when a few drops of sudan III were added, the globules were stained red; in addition, the red blood cells were hemolyzed and destroyed. There was a close resemblance between the action of Lysol on the blood in vitro and the action of the chemicals injected into the uterus on the blood in the intrauterine sinuses.

The police were able to elicit from friends of the dead woman only the statement that she had taken an injection on the day of her death in order to terminate her pregnancy. The time of the injection, the material injected and the amount could not be ascertained.

CONCLUSION

The clinical history, the necropsy and the various laboratory examinations indicate with a reasonable degree of certainty that Lysol, or a preparation similar to it, was injected into the uterus to induce abortion. Before the abortion occurred, the injected solution entered the venous sinuses of the uterine wall and caused death by a combination of severe pulmonary oil embolism and toxic action of the cresol and alkaline components of the injected solution. The depression of the central nervous system, the hemorrhages in the renal tubules, the destruction of the blood and the leukocytic collections in the smaller blood vessels were referable to the irritant and toxic action of the solution injected and the pulmonary symptoms were due to the oil embolism.

COMMENT

Fatal oil embolism has resulted from the injection of various oils into a hollow viscus which possesses a network of rather large veins just under the mucous surface; in such instances the veins were traumatized in such a way that a large portion of the injection mass entered their lumens. Patterson [1] described a case in which death occurred after liquid petrolatum had been injected into the urethra for the purpose of dilating a stricture. Carr and Johnson [2] reported a death from pulmonary oil embolism, which developed after 50 cc. of sterile cottonseed oil had been injected into the bladder in connection with catheterization.

The pregnant uterus, because of the increased vascularity of its wall adjacent to the fetal membranes, offers a ready portal of entry to any liquid substance injected between the amniotic sac and the uterine wall. In the year 1932, oily pastes and salves, such as Leunbach's Paste, Antigravid, Provokol and Interruptin, were injected into the uterus to produce therapeutic abortions,[3] and in some cases death from oil embolism of the pulmonary type was reported as one of the complications of such procedures. Reuter [4] described a death of this type following injection into the pregnant uterus of salve containing tincture of iodine and liquid petrolatum among other ingredients. Brack [5] reported pulmonary oil embolism occurring in 2 pregnant women after intrauterine injection of

1. Patterson, E. A.: J. A. M. A. **97**:1147, 1931.
2. Carr, J. L., and Johnson, C. M.: J. A. M. A. **104**:1973, 1935.
3. Abortifacient Pastes, report of the Bureau of Investigation, J. A. M. A. **98**:2155, 1932. Abortion and Leunbach's Paste, editorial, ibid. **111**:535, 1938.
4. Reuter, K.: Med. Klin. **28**:1339, 1932.
5. Brack, E.: Zentralbl. f. Gynäk. **56**:122, 1932.

Interruptin. The dangers of this method of terminating pregnancy soon became evident, so that now it is not any longer considered a legitimate method of inducing abortion.

The intrauterine introduction of Lysol as an abortifacient has been referred to briefly in the literature. Witthaus and Becker [6] mentioned 3 cases with one death, but did not give any further details. Prüssmann [7] noted that Lysol injected into the uterus of a nonpregnant woman caused her death a few days later from pelvic peritonitis and that a pregnant woman treated by intrauterine injection of Lysol died later of general peritonitis.

Hammer [8] reported the case of a 30 year old multipara whose uterus was flushed out after her delivery with large quantities of 1 per cent Lysol solution as a prophylaxis against puerperal infection. The treatment was continued, and on the eighth day severe dyspnea developed, and two days later the patient went into a state of collapse and died; pulmonary embolism was suspected as the cause of death. The necropsy disclosed a thin layer of necrotic pseudomembrane lining the uterine cavity and general signs of Lysol poisoning, but pulmonary embolism was not observed. The possibility of pulmonary oil embolism was not considered.

The case reported by Straus and De Nosaquo [9] is interesting in demonstrating the action of a paste known as Utra-jel, which was injected into a pregnant uterus as an abortifacient. This preparation contained castor oil–potassium hydroxide soap, pine oil, alkali-combined iodine and water; its action on the blood in the uterine venules and on the blood in vitro was similar to the reactions of Lysol in my case. Utra-jel produced laking of red blood cells, formation of granular pigmented masses and enmeshing of globules of oil in these masses. Death occurred when the uterine wall was perforated by the corrosive action of the paste, with development of suppurative peritonitis. Pulmonary oil embolism was not observed.

I have not been able to find in the literature any reference to a case of pulmonary oil embolism following an injection of Lysol made into the pregnant uterus. The noteworthy features in my case are the absorption of the drug into the uterine veins, the destruction of the blood and the inflammatory changes in the organs caused by the alkali in the injected solution, and the precipitation of the oil globules from the solution, part of which were imprisoned in the transformed blood and part of which were transported to the lungs, causing the pulmonary oil embolism. The embolism was severe enough to embarrass the pulmonary circulation and this, combined with the toxic action of the cresols on the central nervous system and the heart muscle, was sufficient to cause death in a few hours.

6. Witthaus, R. A., and Becker, T. C.: Medical Jurisprudence, Forensic Medicine and Toxicology, New York, William Wood & Company, 1911, vol. 4, p. 1187.

7. Prüssmann: Zentralbl. f. Gynäk. **29**:462, 1905.

8. Hammer, F.: München. med. Wchnschr. **50**:897, 1903.

9. Straus, R., and De Nosaquo, N.: Arch. Path. **39**:91, 1945.

SUMMARY

Lysol injected into a pregnant uterus as an abortifacient caused death as a result of pulmonary oil embolism and Lysol poisoning combined.

PAPILLARY CARCINOMA ARISING FROM RETE TESTIS

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In the testis one rarely has the opportunity of observing neoplasms before they have become so massive that the possible point of departure is totally obliterated. Recently we were struck by the unusual gross appearance of a testicular tumor and were most fortunate in finding histologic evidence pointing almost certainly to the epithelium of the rete tubules as the site of origin. Furthermore, in our experience the clearcut papillary character was in itself a unique feature and set us in search of similar examples in the literature.

Saint-Donat,[1] 1696, appears to have been the first to mention testicular tumors. Subsequently Cooper [2] in 1845 and Johnson [3] in 1856 wrote on the subject. Curling [4] in 1854 made the first reference to an origin in the rete testis in these words: "It being clear that neither the tubuli seminiferi nor the ducts of the epididymis are the tubes which undergo these changes, constituting cystic disease, its seat may be considered as conclusively traced to the ducts of the rete testis."

Much of the later history revolves about the question as to the teratomatous or nonteratomatous origin of male gonadal tumors. Chevassu [5] expounded and championed the dualistic view, while Ewing,[6] after reviewing Wilms's [7] and Langhans' [8] articles, supported the unitarian theory of origin. Gordon [9] state that the problem was "whether practically all tumors of the testis are of teratomatous origin or whether neoplasms may arise by anaplastic changes from adult germinal epithelium, without evidencing totipotent qualities." Bell [10] described possible transition stages to carcinoma, supporting the dualistic theory, but at the same time he implied that the tumor is more likely the result of overgrowth of a single blastodermic element of a teratoma. In Gilbert's [11] case, studied by Ewing, rete and seminiferous tubules seemed to be transformed to carcinoma, but Ewing could not be certain whether this represented direct transformation or invasion. From the foregoing review it appears that at best there exists only

Fig. 1.—Halftone drawing depicting a sagittal surface of the testis and its tunics. *Left:* Obliterating the space between the testis and the tunica vaginalis is a neoplasm, spreading out over the upper pole and slightly invading the now compressed and elongated corpus testis. *Right:* A segment of tunica vaginalis with neoplasm and fibrinous exudate over the inner aspect. The papillary character of the carcinoma is evidenced by some of the uppermost masses.

From the Department of Pathology, University of Oregon Medical School.

1. Saint-Donat: Nouvelles observations sur la pratique des accouchements, Paris, 1715, p. 79; cited by Ewing,[6] by Gordon [9] and by Bell.[10]

2. Cooper, A. P.: Observation on the Structures and Diseases of the Testis, Philadelphia, Lea & Blanchard, 1845.

3. Johnson, G.: Med.-Chir. Tr. **42**:15, 1859.

4. Curling, T. B.: Med.-Chir. Tr. **36**:449, 1854.

5. Chevassu, M.: Tumeurs du testicule, Thesis, Paris, no. 193, 1906, p. 130.

6. Ewing, J.: Surg., Gynec. & Obst. **12**:230, 1911.

7. Wilms, M.: Deutsche Ztschr. f. Chir. **49**:1, 1898; Beitr. z. Path. Anat. u. z. allg. Path. **19**:233, 1896.

8. Langhans, T., in von Billroth, T., and Luecke, G. A.: Deutsche Chirurgie, Stuttgart, F. Enke, 1887, no. 50B, p. 414.

9. Gordon, W.: J. Urol. **43**:851, 1940.

10. Bell, F. G.: Brit. J. Surg. **13**:7 and 282, 1925.

11. Gilbert, J. B.: J. Urol. **43**:722, 1940.

uncertain evidence of a primary origin of carcinoma within the rete testis.

Papillary adenocarcinoma of the testis has been observed previously, although for the most part it has proved to be a one-sided overgrowth of a teratoma. Some of the instances of papillary carcinoma when tracked down to the original papers have been found described only with respect to clinical aspects or so inadequately described pathologically that for our purpose they are worthless.

Fig. 2.—Photomicrograph of rete and seminiferous tubules. At and to the left of center are masses of the neoplasm, the papillary character of which is best illustrated by the intratubular growth in the upper left corner. All foci of carcinoma are within rete tubules. An uninvolved tubule is situated between the two largest accumulations of neoplasm.

In spite of Ewing's [12] statement that teratomatous elements are present in all cancerous testicular neoplasms and may be found if searched for with sufficient diligence, it is our belief that such is not the case in the example seen by us.

REPORT OF A CASE

Clinical Summary.—C. K., aged 59 years, white, married, was admitted to Multnomah County Hospital on Sept. 26, 1944, with chief complaints of a mass in the left side of the scrotum, left-sided hemiplegia and urinary incontinence.

The scrotal mass, first noticed two years previously, had progressed steadily in size and had been aspirated twice by the family physician. Aside from the fact that the fluid obtained was clear, the patient knew nothing more about it. The swelling was not tender or painful. The hemiplegia, of two months' duration, and the urinary incontinence associated with it, were given as reasons for coming to the hospital.

Examination confirmed the presence of left hemiplegia and a swelling of the left half of the scrotum which seemed to be mainly posterior to the testis, particularly superiorly. The exact size of the mass could not be determined. It was fluctuant, but the record does not state whether it was translucent. The right testis was unchanged.

With a preoperative diagnosis of hydrocele, the left testis and tunica vaginalis were extirpated, displaying what appeared at that time to be a hematocele.

Subsequently, personality changes developed, and it was planned to transfer the patient to another hospital for psychiatric treatment, but on November 16 he died from uncertain cause, and permission for necropsy was refused.

12. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 860.

A roentgenogram of the chest two days after admission failed to show anything abnormal in the lungs or the heart. The urine was not tested for sex hormone levels.

Macroscopic Description of Testis.—Before being sent to the laboratory the specimen had been bisected. In this somewhat distorted state it had a length of 11 cm. The other dimensions could not be ascertained accurately. After fixation, a midsagittal section revealed a large mass of fibrin and dark blood clot in the space between the tunica vaginalis and the tunica albuginea. Over the upper pole, however, the fibrin and the blood gave way to a yellowish, often cauliflower-like and distinctly friable tissue, from 5 mm. to 2 cm. in thickness. Outside this, the white tunica vaginalis stood out sharply everywhere, and no neoplasm extending through this tunic was demonstrable. Over a goodly part of the posterior aspect the entire space between the tunics was occupied by the tumor (fig. 1). Only over the anterior surface of this testis was there neither fibrin nor neoplasm in the sac.

The corpus testis was considerably elongated, and its lower pole was fairly sharp and pointed, giving the impression that the alteration in form was due to compression from without. The amputated end of the vas deferens was identified and when followed downward became incorporated in a mass of tumor tissue which totally obscured the epididymis. Over the upper pole of the corpus testis the tunica albuginea was lacking for a short distance, and here the neoplasm, so abundant above the testis, invaded the corpus over an area of about 8 by 8 mm. At no other point along multiple sagittal sections was there any sign of involvement of the body of the gonad, which was soft, hyperemic and compressed only in the manner previously described.

Microscopic Description.—Sections cut to include the mass at the upper pole of the testis proved to contain the epididymis, the tunica vaginalis and the tunica albuginea. Filling the space between the tunics, invading both, and to a lesser degree the intertubular connective tissue of the epididymis, was a neoplasm showing a striking tendency toward papillary arrangement. There was no evidence of any distinct point of origin of the new growth in this region. The involvement of the epididymis was plainly from the outside, leaving the mucosa unaffected. Spermatozoa were lacking in these tubules. On occasion the tumor cells lay in capillary vessels, which, from the absence of blood, one would surmise were lymphatics.

Sections from other large blocks, taken from a point directly inferior to the first and known to include the superior pole of the corpus testis, displayed not only testicular tubules but also tunica albuginea and a segment of rete testis. The mass observed macroscopically in the corpus proved to be neoplasm. As in the epididymis, the growth in this location was between the tubules and never within them or in any way connected with their cells. Spermatogenesis was incomplete and differentiation beyond spermatoblasts could not be demonstrated. The tunica albuginea now appeared as a frayed-out zone and had been interrupted by the carcinoma.

Of the greatest interest were the tubules of the rete. Here quite a number of original and still readily recognizable tubules remained. On the other hand, there were tubules of rete type that were partially filled by new growth and thus could be identified positively as rete (fig. 2). Still other tubules were filled completely by papillary neoplasm. Masses of new growth also lay between the tubules. Of the greatest importance in establishing the origin of the tumor from the epithelium of rete tubules was the fact that at several points the normal cells suddenly gave way to simple or complex papillary overgrowths. It is also of interest that the epithelium covering the branching strands was almost indistinguishable from the parent cells where no growth was seen. It was only in the more solidly cellular areas of the neoplasm that occasional mitoses occurred and that the cells tended to be columnar or spindle shaped. Still another evidence of the cancerous nature was invasion of vessels, one of which could be definitely recognized as a small blood capillary.

Both of the tunics were thickened by reason of the laying down of young connective tissue in the presence of a slight extension of the neoplasm in them, together with hemorrhage.

Pathologic Diagnosis.—Papillary carcinoma, arising in the rete testis, with invasion of the tunics, the corpus testis, the epididymis and the vascular channels; hematocele secondary to carcinoma; aspermatogenesis.

COMMENT

Any one familar with testicular neoplasms will be impressed by the great differences between the tumor herein described and the only type of cancer commonly seen in the male gonad, namely, embryonal adenocarcinoma or seminoma.

In our case there was minimal, not maximal, involvement of the corpus; the testis, although compressed by the tumor, had not been pushed aside until only a thin remnant remained; the new growth was largely outside the testis and in the sac surrounding it, quite in contrast to embryonal adenocarcinoma, which penetrates the tunica albuginea only when very advanced, if at all.

It might be argued that the cell of origin was not epithelial but mesothelial and that the growth arose from the tunics, or that, if the cell of origin was epithelial, the epididymis was the point of departure. Or it might be said that as a result of a one-sided growth a teratoma became a papillary carcinoma. Against all of these possibilities are the observed fact of transition from preexistent rete tubular epithelium to papillary carcinoma and the lack of evidence of any such involvement in other types of tubules. Furthermore, if the tumor were of teratomatous origin, the thorough search made should have disclosed other cell elements somewhere, and it did not.

The gross characteristics of the tumor were such that one must believe that it arose so near

to the space between the tunics that extension to the sac occurred early, giving rise to hydrocele of nearly two years' duration and later to hematocele, and that not until late in the course of its existence did it penetrate into the corpus testis. Anatomically, the rete testis is so situated that just this sort of extension is possible. Not of necessity would a carcinoma, particularly one that even when well advanced did not obstruct all rete tubules, have to invade the corpus and produce complete atrophy of the seminiferous tubules.

In view of the fact that the subject lived less than two months after removal of the testis, that he entered the hospital because of hemiplegia and that personality changes came into the clinical picture, it is regrettable that autopsy was refused. One cannot but wonder whether cerebral metastases were responsible for the involvement of the central nervous system.

SUMMARY

In an unparalleled instance of testicular carcinoma, the origin was satisfactorily traced to the epithelium of the rete testis. The clinical history strongly suggests that the neoplasm was present for at least two years. The bulk of the tumor was situated outside the corpus testis, and the evidence indicates that this portion of the testis was not involved until late in the course of the tumor.[13]

13. Since the writing of this paper there has appeared in the ARCHIVES OF PATHOLOGY (**40**:68, 1945) a paper by Bert E. Stofer, describing a testicular tumor in part cystic and resembling rete testis and in part "peritheliomatous." He related the origin of this tumor to adult seminiferous epithelium and cited similar examples from the literature. In his case and some of those in the literature the maximum point of involvement was the corpus testis. Our case is quite dissimilar except for the long duration of the tumor and the papillary structure.

THROMBOSIS OF THE GASTRIC CORONARY VEIN WITH SPONTANEOUS RUPTURE OF THE STOMACH

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The unusual site of the venous occlusion, the lack of known contributing factors in its genesis, the rapidity of its sequels and the absence of any description of a similar case in the literature review have prompted this report.

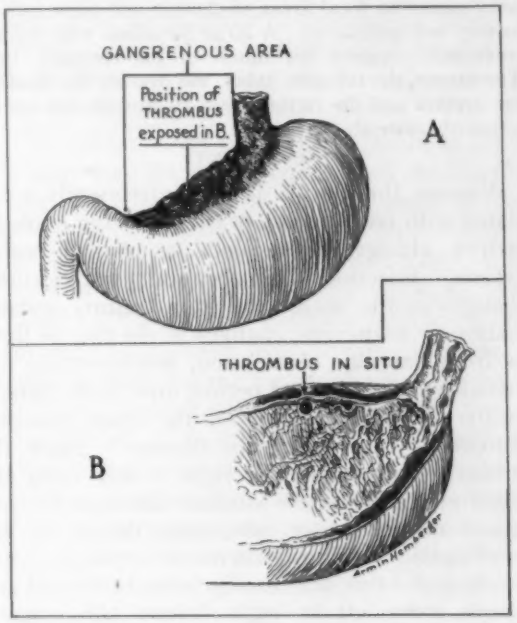
REPORT OF A CASE

The patient was a 56 year old white unmarried woman who was admitted to the Emory University Hospital in a state of extreme shock with the diagnosis of "acute disease of the abdomen," made by a local physician.

According to the nearest relative, the patient had been in fairly good health until 1 p. m. on the day of admission. After a normal morning's work as the local postmistress, she ate lunch as usual. Immediately thereafter she suddenly experienced acute excruciating pain in the midepigastric region. This was soon followed by severe nausea, vomiting and prostration. On arrival at the hospital, seventeen hours after onset of the illness, she was in a comatose state. After further questioning, the family stated that the patient had complained of attacks of "indigestion" in the past. The rest of the history was irrelevant.

The temperature was 99.4 F., the pulse rate 100, the respiratory rate 26 and the blood pressure 60 systolic and 30 diastolic. The patient was fairly well nourished, approximately of the stated age, acutely ill, in extreme shock and comatose. She constantly expelled a brown vomitus of fecal character. The peripheral vessels were collapsed, the pulse barely palpable. The pupils were constricted. Auscultation of the chest revealed numerous coarse gurgles throughout, with barely audible heart sounds. The abdomen was markedly distended and of boardlike rigidity; no peristalsis was heard. Urinary and fecal incontinence was present. The impression was ruptured peptic ulcer with peritoneal involvement, diffuse mesenteric thrombosis or fulminating pancreatitis. Therapy consisted of maintaining the position recommended for a patient in shock, application of administration of warmth, administration of nickethamide, nasal administration of oxygen, injection of 2 units of plasma and injection of 5 per cent dextrose in saline solution. The patient became cyanotic and died nine and three-quarter hours after admission.

Necropsy.—The body was that of a fairly well developed, well nourished, 56 year old white woman, diffusely cyanotic. Rigor and livor mortis were minimal. The examination was made two hours after death. The thoracic wall was emphysematous; the abdomen, markedly distended and tympanitic, with definite flattening of the umbilicus. No edema or abnormal discoloration of the subcutaneous tissues was noted. The peritoneal cavity revealed marked gastrointestinal distention, with 4,325 cc. of brownish fluid contained in the stomach. In the distal portion of the lesser curvature the surface of the stomach revealed an area 1.5 cm. in diameter covered with a thin film of coagulated blood. No perforation was observed at this site. In addition there was an area of gangrene 8.5 cm. in length in the upper third of the lesser curvature of the stomach. At numerous points the transverse colon and the duodenum were adherent to the under surface of the liver. The pleural cavity and the lungs were grossly normal except for some basal atelectasis. Partial alveolar collapse and hypostatic congestion were seen microscopically. The heart weighed 200 Gm. and showed no definite lesions. Sclerosis of the coronary arteries and moderate perivascular fibrosis were observed on further examination. In the ascending portion of the aorta were patchy areas of atherosclerosis. The liver, the gallbladder and the duct system were grossly normal. Microscopically,



A, area of gangrene extending along the lesser curvature of the stomach. *B*, site of rupture with the thrombus of the coronary vein in situ.

the hepatic capsule was thickened; the liver cells proper showed granular cytoplasm, and there were sinusoidal congestion and periportal mononuclear cell infiltration. The spleen was normal in size, weight and consistency but showed occasional microscopic zones of chronic inflammation. Each kidney (right, 70 Gm., left, 80 Gm.) revealed a moderately adherent capsule, with minimal decortication on attempts at removal. On cut section there was moderate obliteration of the cortical striae, with yellowish linear streaking in the medullary pyramids. The pancreas and the adrenal glands appeared grossly normal. The intestinal tract appeared normal except for moderate distention of the ascending colon. The superior mesenteric, celiac, portal and

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vena cava vessels revealed no abnormalities. The stomach (*A* in the figure) was markedly distended, and the previously described area of gangrene was seen extending along its lesser curvature down to within 4 cm. of the pylorus. It was about 1.5 cm. in width, and in its middle portion there was a soft, necrotic, ragged perforation measuring 0.6 cm. in width. The largest radicle of the coronary vein (*B* in the figure) situated in this area was completely filled by a fairly recent antemortem reddish gray clot 4 mm. in length and 5 mm. in width. The wall of the vein showed evidence of some intimal damage, since it was irregular and roughened. Microscopically, the gastric mucosa was partially ulcerated and eroded in some areas. The marginal tissue was edematous and contained numerous granulocytes (polymorphonuclear and eosinophilic) and lymphocytes. A zone of fibrinoid necrosis with scattered nuclear debris was seen occupying the area subjacent to the purulent exudate. The submucosa and muscularis were edematous and infiltrated with polymorphonuclear leukocytes. The capillaries and the arterioles of the submucosa were dilated and filled with erythrocytes. The muscular coat showed moderate necrosis, and throughout the serosal layer there were numerous focal areas of chronic and acute inflammatory cell infiltration. A large thrombus with definite lamination occupied the lumen of the coronary vein. The uterus, the fallopian tubes, the ovaries, the bladder, the urethra and the rectum were all grossly and microscopically normal.

COMMENT

Venous thrombosis is not infrequently associated with one or more of the following etiologic factors: changes in the blood, as from thrombocytosis, infection or chemical intoxication; changes in the vessels, as from trauma, inflammation or aneurysm; changes in the rate of flow, as from vascular obstruction, postoperative immobility and the agonal period, and finally defects in the endothelial lining of the blood vascular system, as from injury or disease.[1] Since the coronary vein runs from right to left along the lesser curvature of the stomach between the two layers of the lesser omentum, thence to the esophageal opening, where it receives some esophageal veins, and finally turns backward and passes from left to right behind the omental bursa to end in the portal vein,[2] the possibility of primary pyelophlebitis was seriously considered in this case. Subsequent dissection revealed nothing significant in the portal system. Moreover, the history of symptoms like those of gastric ulcer was seriously considered in connection with the genesis of the thrombotic process, but gross and microscopic examination of serial sections of the entire esophageal and gastric mucosa revealed no evidence of ulcer. No history or physical evidence of abdominal trauma was obtained.

Occlusion of the vessels supplying the gastrointestinal tract is most frequent in the superior mesenteric system[3] (90 per cent) and in the superior mesenteric arteries (60 per cent). The vessel usually is the seat of moderate arteriosclerosis, and the obturation is caused by a thrombus. Soon after the vascular occlusion, the viscus becomes dark red and then deep purple. The serosa is dull and frequently covered by a fine fibrinous exudate. With the passage of time, the wall increases in thickness, largely because of edema and hemorrhage. The mucosa undergoes autolysis, and finally the entire wall changes into a friable, dark red, soft tissue. Clinically this may be characterized by a sudden onset with signs of peritoneal irritation and intestinal obstruction. Unusual sites of intestinal infarction in which the omentum was involved have been described by Lipsett[4] and Pines and Rabinovitch.[5] In the former's case torsion was the causative factor, and in the latter's the infarction was segmental and its cause unknown. In addition, Eliason and Johnson[6] have reported this same process in the appendices epiploicae. As far as can be determined from the literature, there is no reported evidence of gastric venous or arterial thrombosis.

SUMMARY

A spontaneous rupture of the lesser curvature of the stomach was due to thrombosis of the coronary vein with subsequent local gangrene.

1. Babcock, W. W.: Principle and Practice of Surgery, Philadelphia, Lea & Febiger, 1944, pp. 32-33.
2. Lewis, W. H.: Anatomy of the Human Body, Philadelphia, Lea & Febiger, 1930, p. 682.
3. Lazarus, J. A.: Am. J. Surg. **33**:129, 1936.
4. Lipsett, P. J.: Ann. Surg. **114**:1026, 1941.
5. Pines, B., and Rabinovitch, J.: Surg., Gynec. & Obst. **71**:80, 1940.
6. Eliason, E. I., and Johnson, J.: Surgery **6**:68, 1939.

CRUVEILHIER-BAUMGARTEN DISEASE

Splenomegaly, Portal Hypertension and Patent Umbilical Vein

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Cruveilhier-Baumgarten disease and the more inclusive clinical syndrome have been known for many years. With the original reports of Cruveilhier in the years 1833 to 1835 and Baumgarten in 1908 there have accumulated in the foreign literature many references and reports on this clinical syndrome and disease. The first direct reference to the condition in the English literature appeared in 1942, when Armstrong and associates [1] reviewed the entire foreign literature, from which they tabulated the data on 52 previously reported cases, and they added 3 cases. Of these 55 cases, 6 fell into the group of Cruveilhier-Baumgarten disease. The remaining cases were examples of the clinical syndrome. The second reference to appear is that of Valk and Horne,[2] who reported in some detail the fifty-sixth case of the syndrome. The third reference is that of Yater and Kendrick,[3] who reported 2 additional cases of the syndrome, bringing the total number now reported to 58, of which 6 appear to represent true examples of the disease.

The Cruveilhier-Baumgarten syndrome is characterized by an enlarged spleen, a small or unenlarged liver, distended abdominal veins, over which a venous thrill or murmur can be heard, and persistent leukopenia. This syndrome can occur in any case of portal hypertension, such as portal cirrhosis, portal thrombosis or Banti's disease, in which recanalization of the umbilical vein results in shunting of large amounts of blood from the portal vein through the umbilical vein to the epigastric veins in the abdominal wall. In rare instances the umbilical vein has remained patent from birth, and it is these instances that are examples of true Cruveilhier-Baumgarten disease. Cruveilhier originally thought that the shunting of large amounts of blood around the liver through the congenitally patent umbilical vein resulted in atrophy of the liver through disuse. Baumgarten was unable to accept this explanation of the small liver and offered the hypothesis of congenital hypoplasia of the portal system, for which there is some experimental evidence.[1] Cruveilhier-Baumgarten disease, then, is characterized by evidence of a congenitally patent umbilical vein and a small or unenlarged liver with an insufficient amount of portal cirrhosis to explain the marked portal hypertension in addition to the other features characterizing the syndrome.

The embryology of the development of the umbilical vein, the portal system and the liver was extensively reviewed in the first paper by Armstrong and associates.[1] It was briefly reviewed in the report by Yater and Kendrick. I shall reiterate here only the fact that while about two months after birth the umbilical vein atrophies to form the ligamentum teres, in a few instances the upper portion of the umbilical vein will remain patent into adult life; this is designated as the Rest-Kanal of Baumgarten. The fibrosed remnant of the umbilical vein has no functional significance unless some disease process resulting in portal hypertension recanalizes it so that a collateral circulation develops through the umbilical vein to the epigastric veins in the abdominal wall.

REPORT OF A CASE

A 43 year old white man was admitted to the United States Marine Hospital in Baltimore with a history of recurrent episodes of hematemesis, necessitating several hospitalizations, and vague epigastric pains of two years' duration. He was a moderately well nourished white man, weighing 155 pounds (70 Kg.), with a blood pressure of 128 systolic and 84 diastolic. The significant physical findings included large, dilated veins in the upper abdominal wall and an enlarged, tender spleen, palpable 3 fingerbreadths below the left costal margin. The liver was not palpable, there was no ascites, and there was no evidence of jaundice.

Roentgenograms of the esophagus and the stomach were reported normal. Serologic study of the blood gave normal results, and the urine was normal. The

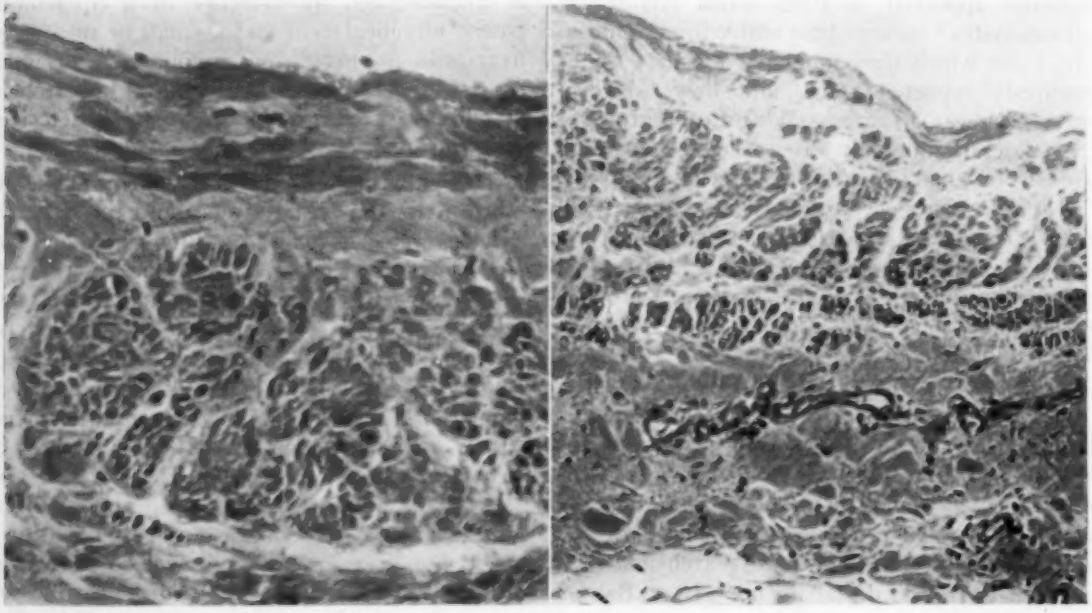
From the Department of Pathology, United States Marine Hospital.

1. Armstrong, E. L.; Adams, W. L.; Tragerman, L. J., and Townsend, E. W.: Ann. Int. Med. **16**:113, 1942.

2. Valk, H. L., and Horne, S. F.: Ann. Surg. **116**:860, 1942.

3. Yater, W. M., and Kendrick, J. P.: M. Ann. District of Columbia **13**:319, 1944.

red blood cell count was 5,000,000, and the hemoglobin content was 13.9 Gm. The white blood cell count was 4,200, with a normal differential count, including 2 per cent eosinophils. The total serum protein was 6.9 Gm., with 4.3 Gm. of albumin and 2.6 Gm. of globulin per hundred cubic centimeters. The result of the cephalin flocculation test for hepatic function was reported as 2 plus and again as 3 plus. The blood cholesterol was 193 mg. per hundred cubic centimeters. The results of fragility tests, the prothrombin time and the bleeding and clotting times were normal. Repeated examinations of the stools revealed no blood. After considerable study and debate, the patient was operated on and the coronary vein at the junction of the esophagus and the stomach ligated. At operation the spleen was judged to be about eight times the normal size and the liver reduced to about one third of its normal size. Biopsy specimens were taken from both the spleen and the liver. Sections of the liver showed leukocytic infiltration with but slight evidence of cirrhosis. The spleen showed large, dilated blood spaces with much fibrosis, not unlike the picture in Banti's disease. These findings suggested the possibility of Cruveilhier-Baumgarten disease; so haste was made to reexamine the abdomen for the presence of a thrill or murmurs; this had not been done before. However, the patient was in profound shock and died a few hours later from recurrent hemorrhages in the gastrointestinal tract. When the patient was examined no definite thrill or murmur could be detected. In this connection, however, it is interesting to note that Yater records in his article that in 1 of his cases, which he followed closely, the murmur disappeared entirely several hours before death. At autopsy, about 2,000 cc. of yellowish fluid was present in the peritoneal cavity, and the spleen was markedly enlarged, weighing 650 Gm., with a very firm pulp. The liver was reduced in size and weighed 1,240 Gm. Most of this decrease was in the right lobe, for the left lobe appeared practically normal in size. Although the entire surface was quite irregular, the liver cut with practically the normal amount of resistance, and the cut surface simply appeared lighter and more yellow than normal. The umbilical vein was large and patent with an internal diameter of 1 cm. when distended. It ran from the portal vein within the liver to a dilated venous pool beneath the umbilicus. A ruptured small gastric varix was found in the cardiac portion of the stomach from which there had been bleeding. There were many dilated esophagleal varices. Microscopically, the spleen showed enlarged, prominent blood spaces, which were mostly empty, with much diffuse fibrosis and large, thickened trabeculae. The lymphoid tissue was markedly reduced, and there was considerable scattered hematogenous pigmentation. Many sections of the liver showed only a mild amount of portal fibrosis in a few scattered areas. In general, the structure of the lobules appeared fairly normal, although a few were perhaps somewhat reduced in size. There was no evidence of hepatic regeneration, and about the central veins in many of the lobules the cells showed collections of small fat droplets. There was considerable diffuse lymphocytic infiltration, particularly in the portal areas. Numerous sections of the umbilical vein showed a large lumen, and the wall was composed of bundles of muscle fibers, both longitudinal and cross cut. Some sections showed small amounts of surrounding fibrous tissue, but the histologic structure indicated that this vein had remained patent from birth and had not been recanalized.



Sections of the patent umbilical vein showing the prominent, well developed bundles of muscle fibers.

SUMMARY

Cruveilhier-Baumgarten disease clinically is characterized by an enlarged spleen, a small or unenlarged liver, distended abdominal veins, over which a venous thrill or murmur can be heard, and persistent leukopenia. Pathologically it is characterized by a patent umbilical vein which shows no evidence of having been recanalized and with little or an insufficient amount of portal cirrhosis to explain the portal hypertension. The spleen shows evidence of long-standing venous stasis. The case of Cruveilhier-Baumgarten's disease described is the seventh to be reported.

In every case of splenomegaly or of known portal hypertension coming to autopsy there should be a careful examination of the ligamentum teres for a patent umbilical vein. If this examination is invariably made, perhaps Cruveilhier-Baumgarten disease will be found not to be so rare as the present total of 7 reported cases would indicate.

Laboratory Methods and Technical Notes

CARE OF THE BODY AT AUTOPSY IN COOPERATION WITH THE MORTICIAN

T. C. Laipply, M.D., Cleveland

The importance of the autopsy for the progress of medicine has been emphasized in many publications, and so far as physicians are concerned it needs no restatement here. However, in spite of the combined efforts of members of the medical profession, the matter is often not understood by the laity in general and the funeral directors and embalmers in particular. Indeed, the latter group may so oppose the autopsy that influence is exerted to obstruct consent and even to secure withdrawal of consent to the autopsy after it has been granted. This unfortunate attitude is usually predicated on (1) a failure to understand the purposes and the significance of the postmortem examination, (2) undue delays in the performance of autopsies, (3) delays in the preparation of death certificates and (4) failures to prepare bodies properly, and even mutilation of the bodies, by unskilled prosectors. This article offers suggestions which may improve the whole situation.

IMPORTANCE OF AUTOPSIES

There are many reasons for performing such examinations, and in most large medical centers, in seeking permission for autopsies, a great deal of time is spent convincing members of the laity of their value. Rarely, however, is any attempt made to increase the morticians' appreciation of postmortem examinations. Without concerted effort in this respect, cooperation will never be complete. These persons should be made cognizant of the need for autopsies, the amount of work entailed and the manner of performance. Incomplete understanding is prevalent because the procedure has been emphasized as a way of procuring purely scientific knowledge, without direct benefit to relatives, or has been considered as a liberal concession to the forgivable curiosity of a beloved family doctor. Particularly among morticians it is a popular misconception that autopsies are done for the sole benefit of the pathologist or an assistant who desires to perfect his skill in dissection. It should be pointed out that the work is done by, or under the supervision of, a trained pathologist, not only for the advancement of science but also to elevate and maintain standards of medical practice. Attention should be directed to the fact that every one, including the mortician, has an equal chance of benefiting by such examinations, which serve as the layman's best guarantee of good medical treatment. Physicians realize that the autopsy serves as an enlightening and stimulating means of promoting diagnostic acuity. The clinician who never has the opportunity of being corrected at the autopsy table is deprived of a powerful incentive to increased knowledge and competence. In view of these facts it is difficult to understand how informed persons, in particular doctors of medicine, can justify not having actively requested the performance of autopsies on themselves or relatives for whom they have acted as responsible agents.

It should be equally evident to all physicians, including pathologists, that complete autopsies are more desirable than limited ones. If discoveries are to be made, it is essential that the unexpected be looked for. If the pathologist examines only the organs which the clinician suspects of being diseased, the stark corrective nature of the procedure is lost.

UNDUE DELAY IN THE DELIVERY OF BODIES TO FUNERAL DIRECTORS

Unnecessary delay in the delivery of bodies to funeral directors has caused more ill-feeling among morticians than any other single factor in connection with the performance of autopsies. The two factors which materially retard the release of bodies to funeral directors are delay in performance of the autopsy and failure promptly to prepare the death certificate.

The performance of an autopsy as soon as possible after consent is obtained is the duty of the pathologist. The removal of the organs en masse is helpful in facilitating prompt repair of the body. In many institutions adequate provisions have been made by the pathologist to assure completion of autopsies without undue delay. It is, however, a startling realization that in many highly regarded hospitals there is no systematized procedure that is adequate and workable for the preparation of death certificates. Even in well organized hospitals it is not uncommon for funeral directors to be compelled to wait from one-half hour to as much as three hours for a physician, engaged in the operating room or otherwise, to sign the death certificate. The guilt for the existing inade-

From the Institute of Pathology of Western Reserve University and University Hospitals of Cleveland.

quate systems rests with the hospital administrators and must be corrected if relations are to be improved.

CONSENT FOR AUTOPSY

The laws concerning dead human bodies and governing coroners and medical examiners in the United States have been covered in Bulletins of the National Research Council.[1] The physician in charge of the patient, the officers of the hospital or other institution, and the pathologist should be familiar with the laws, at least with those in their own state. A written and properly witnessed authorization should be obtained from the lawful custodian of the body, unless the examination has been ordered by the coroner or the medical examiner. The one lawfully authorized to grant the permission is ordinarily the next of kin. The rights of kinship are vested first in the surviving spouse. If there is none, lawful custody belongs to reletives in the following order: children of the deceased, then parents, then brothers and sisters, and so on. The rights of relatives of equal degree of kinship, such as brothers and sisters, are not fixed by law. In most instances, however, one of these assumes custody of the body and is entitled to give consent. In such instances, a dissenting member of the group would probably have difficulty proving that he had suffered a trespass of his rights. In order to prevent misunderstandings and even legal entanglements, it would seem advisable for all hospitals in any given community to adopt the same form of recording permission for an autopsy.

The form illustrated in figure 1 has proved satisfactory and can be recommended. The various parts of the body which may be examined are listed so that nothing will be overlooked; if consent is not obtained for all, the rest are deleted by the physician. Furthermore, the pathologist is authorized specifically to remove organs for subsequent study. Although such removal is permitted by common law, this provision is advisable. Important is the requirement that a physician inspect the body after its repair.

(Front)

UNIVERSITY HOSPITALS OF CLEVELAND

Cleveland, Ohio.....

I do hereby grant permission to the authorities of University Hospitals of Cleveland to perform an autopsy on the body of ....., a deceased patient, with the object of ascertaining the direct and indirect causes of death, including such examination of thorax and abdomen, brain, spinal cord, peripheral nerves, bones and marrow, neck, and organs of special senses, as may be necessary for this purpose, and to remove such parts of the body as may be deemed necessary for study subsequent to the autopsy.*

Signed .....

Witness to signature

Relationship to Patient.....

Address .....

.....

Approved

Administrative Officer

NOTE: This form must be completely filled out on both sides, signed in DUPLICATE and filed at the information desk. Ink or indelible pencil must be used in completing the original. Carbon paper may be used for the duplicate.

* Examination should be deleted for which consent is not obtained.

B-23 1m 5-42U

(Back)

Date of Death.....(Hour).....

Hospital No.....Age.....Sex .....Color .....

Clinical Diagnosis .....

.....

.....

Remarks .....

.....

Signed.....

Body inspected after repair.....M.D.

The body is to be released to.....

(Undertaker's name and address)

who is authorized to call for same

Date .....

at .....A. M. P. M.

Signature of House Officer.....

Fig. 1.—An acceptable form for the recording of the consent of the next of kin to the performance of an autopsy.

REPAIR OF THE BODY

The proper repairing of the body after autopsy is the responsibility of the pathologist. In most medical centers routine methods have been established to insure satisfactory results. Although improper repair has been somewhat overemphasized as a cause of lack of cooperation between morticians and pathologists, yet even in large medical centers there are occasional examples of inadequacies in this procedure. When the repair is satisfactory, no extra time or effort is required to embalm the body. There is, therefore, no valid reason for the funeral director or embalmer to increase the charge for taking care of a body on which an autopsy has been done. It is, of course, obvious that morticians have certain obligations to fulfil, which may be made needlessly difficult if the care of the body after postmortem examination is unsatisfactory.

1. Weinmann, G. H.: A Survey of the Law Concerning Dead Human Bodies, Bulletin 73, National Research Council, Washington, D. C., 1929; A Compendium of the Statute Law of Coroners and Medical Examiners in the United States. Bulletin 83, National Research Council, Washington, D. C., 1931.

Successful cooperation in this regard is more likely if representatives of pathologists and morticians of the community have occasional meetings to decide on an acceptable method of repair and to correct the faults in the system that are likely to occur from time to time with changes in hospital personnel. The fact that the autopsy from beginning to end is a part of the professional service rendered by the physician should stimulate cooperation on the part of the administrators of hospitals and pathologists and thus prevent negligence in the final care of the body.

Care of the Exterior of the Body.—Before being released to the funeral director the body should be thoroughly cleansed—with a disinfectant if necessary, as indicated in a later section. If blood is washed off before it clots, or soilage is prevented, the difficulty of the final cleansing is reduced. Of particular value in rendering the cleansing of the head less difficult is the use of a hood (fig. 2). Such hoods, made of oiled silk or waterproof plastic, are washable and thus reusable. If such a covering is put in place before the thoracicoabdominal incision is made, the hair and the face will

Fig. 2.—A waterproof hood in place over the head.

not be smeared with clotted blood. This protection is of particular value in regard to women, especially when there is no permission for examination of the head.

Primary Incisions and Their Closure.—To facilitate removal of cervical organs and the tongue without disfigurement, the Y-shaped thoracicoabdominal incision is desirable with both males and females. This type of incision is a requirement in autopsies on sailors. Care should be exercised in regard to females to keep the ends of the upper limbs of the Y below the level of the shoulder joints. If this is not done, a décolleté dress, which is sometimes desired, cannot be used. When such incisions are excessively high, leakage of embalming fluid is liable to occur with soilage of garments in conspicuous locations.

The incision in the scalp made prior to removal of the calvarium and the brain should be made far enough back to be invisible from the front. Care is particularly important when the hair is small in amount. When the hair is long, it should be parted at the site selected for the incision. In sawing through the skull plates, the usual V type of incision should be made, and the line of cut should be made obliquely through the tables instead of at right angles to the surface; this reduces the tendency of the calvarium to slip out of place.

Ordinarily incisions are closed with various types of suture material, depending on the conspicuousness of the suture lines. Heavy white cord is a satisfactory suture material for incisions that are ordinarily covered by clothing. It is not, however, satisfactory for the suturing of manifest incisions like those in the scalp. For this purpose a finer suture material, such as no. 0 cotton thread, should be employed. It should be white or tan for the bodies of white persons and dark brown for the bodies of Negroes. The type of stitch used in closing the incisions is of little consequence except when the incisions are on the face, the neck or the hands, where

Fig. 3.—Design of glass cannulas with rubber tubes. Figure 3 is a modified form of that in an article by A. R. Moritz (Hospitals **12**:78, 1938).

the subcuticular stitch is preferable. In all instances the stitches should be sufficiently close together to minimize leakage.

Many embalmers prefer to open the thoracic and abdominal cavities after postmortem examination and repair of the body. It will thus be found in some communities that by mutual agreement complete suturing of the thoracicoabdominal incision may be eliminated. In such communities, after evisceration, the excess fluid

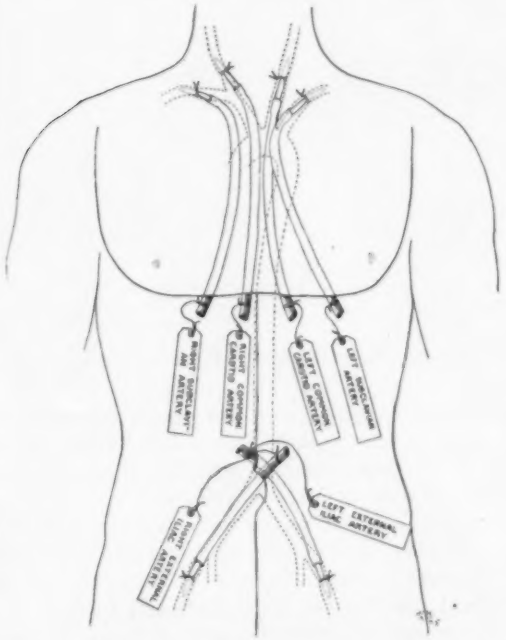


Fig. 4.—The position of the cannulas and rubber tubes after the completion of the repair of the body. Figure 4 is a modified form of that in an article by A. R. Moritz (Hospitals **12**:78, 1938).

is removed, and a small amount of absorbent packing is placed in the cavities. The edges of the thoracicoabdominal incision are then approximated with two or three sutures. It may be desirable to supply an adequate amount of packing for the cavities in a separate bundle.

Cannulas for Arteries.—Particularly if the embalmer does not open the body cavities after the autopsy, his work may be greatly lessened if cannulas with attached rubber tubes are inserted into the cut ends of the large arteries. If they are properly constructed and inserted, the embalmer can attach his pump directly to the rubber tubes and thus embalm the head, the neck and the extremities.

The cannulas can be made from soft glass tubing of moderate thickness. For adults, tubing 7 mm. in diameter is used; for children, tubing with a diameter of 5 mm. is usually satisfactory. The tips of these cannulas may vary in size but are usually 4 and 2 mm. in diameter, respectively. A definite neck near the tip facilitates ligation of the vessel wall about the glass tubing and thus prevents slipping and leakage when the pressure used for embalming is applied. For adults, the glass cannulas are 14 cm. long and the rubber tubes about 20 cm. in length. The smaller cannulas used in children are 8 cm. in length with rubber tubes 16 cm. long (fig. 3).

Such cannulas are placed in the right and left subclavian, the right and left common carotid and the right and left external iliac arteries. The rubber tube from each cannula is extended to the exterior through the primary incision. A label indicating the artery cannulated is fastened to each rubber tube (fig. 4). With such preparation fluid can be injected into these vessels, and at the same time the corresponding portion of the body can be massaged to aid in the flow of the embalming fluid.

The separate cannulation of each of the large arteries is superior to the use of U tubes connected to a single rubber tube because different pressures, as well as massage, may be necessary to inject different parts of the body.

If the procedure outlined is impracticable, ligatures should be placed about the same large arteries. The long ends of the ligatures should extend to the exterior through the primary incision and be appropriately labeled.

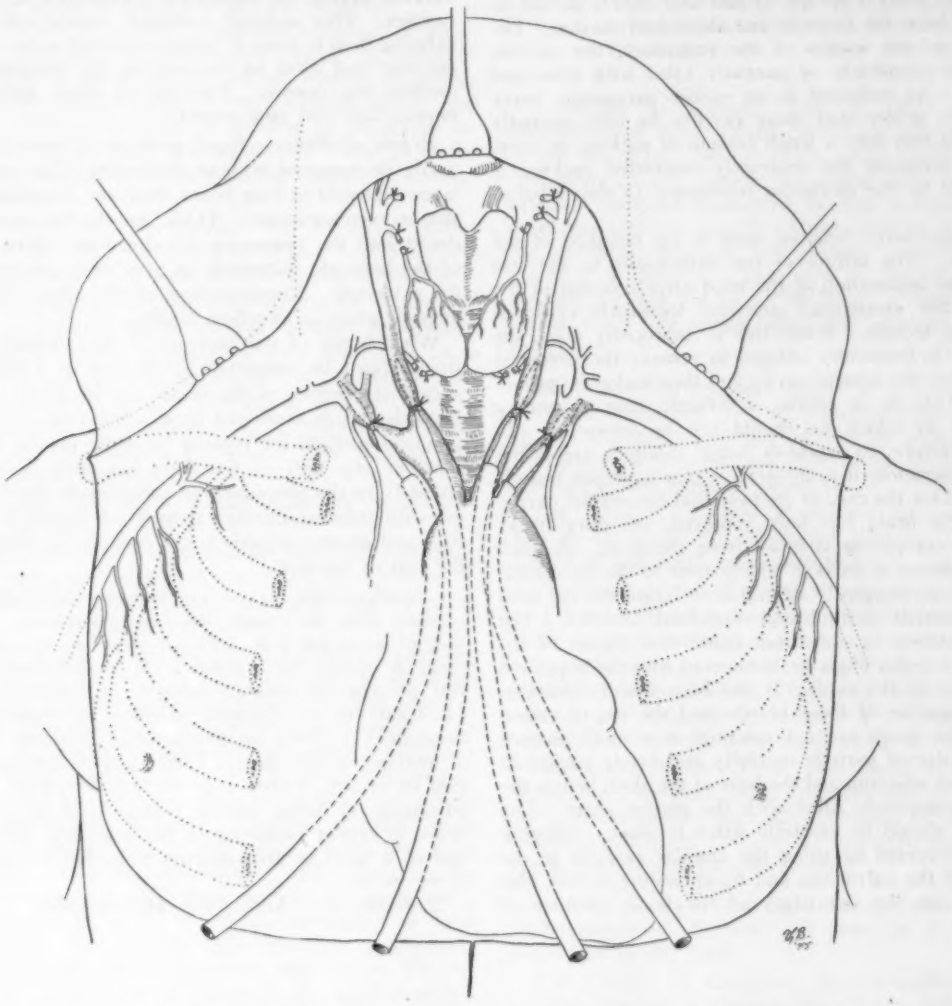


Fig. 5.—The position of the cannulas after the arteries to the neck and the tongue have been cut and tied. Figure 5 is a modified form of that in an article by A. R. Moritz (Hospitals **12**:78, 1938).

Ligation of Arteries.—It is essential to ligate the severed vessels originating from the large arteries through which embalming fluid is to be injected. In general, the following vessels should be ligated:

(*a*) Right and left internal mammary arteries if the thoracic cavity is opened and cannulas are not used;

(*b*) Right and left superior and inferior thyroid arteries if the organs of the neck are removed;

(*c*) Right and left lingual arteries if the tongue is removed;

(*d*) Right and left vertebral and internal carotid arteries if the brain is removed, unless plaster is firmly packed in the foramen magnum and the base of the skull;

(*e*) The vertebral arteries near their origin in the neck if the spinal cord is removed.

Figure 5 shows the cannulas properly inserted in the large arteries. The tips of the cannulas in the subclavian arteries are beyond the origins of the internal mammary arteries, thus obviating the necessity of their ligation.

When the restrictions of the permission prevent removal of the organs from the body, and only small blocks of tissue are excised, the large arteries should be cannulated in the same way.

Care of Body Cavities.—Fluid and debris should be removed from the thoracic and abdominal cavities. Dependent on the wishes of the embalmer, the cavities should be completely or partially filled with absorbent material. As indicated in an earlier paragraph, many embalmers prefer that these cavities be only partially closed and that only a small amount of packing be used. In such instances the separately submitted packing is introduced by the mortician subsequent to the embalming.

The improperly repaired head is the bugaboo of the embalmer. The failure of the pathologist to exercise care in the restoration of the head after removal of the brain is the mortician's principal legitimate criticism of autopsy technic. When this is improperly done, the embalmer is frequently obliged to remove the hardened plaster from the cranial cavity and then make the proper repair. This is, of course, a difficult, time-consuming procedure to which he should not be subjected. In order, therefore, to establish better relations and bring about cooperation, it is imperative that a proper technic be adopted for the care of the head and the cranial cavity.

After the brain has been removed, the dura mater should be completely stripped from the skull. A thick paste of plaster of paris is widely used to fill the cranial cavity. It is frequently advisable to ligate the cut ends of the internal carotid and vertebral arteries. The recommendation is sometimes made that cotton be inserted or wooden plugs be hammered into the foramens in the base of the skull. It has been found, however, that the ligation of these vessels and the use of cotton and wooden plugs are not essential if a small amount of dry plaster of paris is carefully and firmly packed in the foramen magnum and the base of the skull before the cavity is completely filled with the plaster paste. The calvarium should be carefully fitted in place. Slipping may be prevented by using the angular saw-cut in the removal of the calvarium and by spreading a thin film of plaster on the cut edges of the bone. Suture of temporal muscles, which is sometimes of aid, may be a handicap because the sutures are liable to tear out.

Regardless of the method used in reconstructing the skull, there is usually leaking of a small amount of embalming fluid through the incision of the scalp. If the restoration of the skull and the suturing of the scalp have been done with care, this leakage is ordinarily small in amount and of no significance.

If the eyes have been removed, the lids should be sutured together with a small curved needle and fine silk. Balls of paraffin or wax serve as an excellent substitute for the eyeballs. They should be inserted from behind after the lids have been sutured.

In many institutions it is common practice to sprinkle various drying or hardening compounds in the body cavities. This material ordinarily mixes with the embalming fluid to form a sticky mass that serves no useful purpose, and must be removed by the embalmer if the cavities are opened. The use of these substances is unnecessary and undesirable.

Repair of Bones.—Small portions of bones can ordinarily be removed without distortion. The removal of large segments of long bones, however, frequently necessitates reconstruction. Thick, nonflexible wires introduced into the remaining proximal and distal portions of the bone aid materially in preventing the collapse of the extremity. Reenforcement of the wires with plaster of paris gives an excellent result.

When bones of the face are to be removed, the soft tissues must be supported by the use of a cast. Such a cast or positive of the bones can be made of plaster of paris. It is fashioned from a mold or negative. A practical method for making accurate plastic reproductions of any part of the body has been described by Gross.[2] In the procedure, an inexpensive resilient agar-gel-soap-cellulose mixture is used for the mold or negative, and plaster of paris is the constitutive substance of the cast or positive.

Cleansing of the Body.—The body should be thoroughly washed after its repair has been completed. If it is that of a person who suffered from an infectious disease, it should be washed with a disinfectant. For this purpose an aqueous solution of zephiran chloride (1:4,000) or an alcoholic solution of phenylmercuric benzoate (1:2,500) is satisfactory. Aqueous solutions of mercury bichloride (1:1,000) and of saponated solution of cresol (1:50) may be used but have the disadvantage of being strong poisons and irritants. In order to render cleansing of the head less difficult, the use of a hood as described on page 410 is useful.

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2. Gross, P.: Arch. Path. **16**:869, 1933.

Notes and News

Committee on Growth of the National Research Council.—The appointment of a "committee on growth," with membership designed to be broadly representative of the fields concerned in cancer research, both basic and clinical, has already been announced by the National Research Council of the National Academy of Sciences. The committee was created within the Division of Medical Sciences of the Council, as a result of action by the American Cancer Society designating the Academy as its scientific advisor for research.

The committee wishes to call the attention of interested investigators to the general outline of endeavor which it proposes to foster and the general principles by which it will be guided. The committee accepts the interpretation of its field of interest as including reliance on, contact with and support of research in the basic sciences bearing broadly on the whole phenomenon of growth.

The committee has adopted the following major principles by which, so far as possible, it will be guided in its sponsorship of research and training programs:

(*a*) Desirability of long term grants to persons or groups engaged in projects of major importance.

(*b*) Grants, where possible, of such magnitude as to permit individual investigators to appoint associates for long term training periods.

(*c*) Granting of fellowships to institutions for training of workers to acquire new technics and wider experience.

(*d*) Maintenance of continuing individual contact with workers in field.

(*e*) Provision, on a participating basis, for continuing economic security for professional workers.

(*f*) Liberal attitude toward the investigator's work, his publication and reports.

To assist it in the fulfilment of its advisory functions the committee, on its part, will make free use of either ad hoc or standing subcommittees in specific fields of interest. Furthermore, it proposes to arrange conferences of competent groups for discussion of problems, for interchange of reports, etc.; make surveys to analyze problems or to determine progress in areas of special interest pertaining to cancer; evaluate, through study by subcommittees and by the main committee, basic and clinical research undertakings, and submit recommendations for support to the American Cancer Society; initiate and plan broad or specific programs of basic and clinical research, through activities of the subcommittees and main committee, and secure the cooperative efforts of investigators in the general undertakings.

The committee has established a central office in the Washington headquarters of the Council where information on all phases of cancer research will be assembled and from which reports may be distributed to interested investigators.

Many members of the committee have participated intensively in the broad programs of research conducted under the pressure of war. It is both the hope and the sanguine expectation of the committee that the fruitful pattern of cooperative investigations so successfully established during the war years can now be carried on, modified and tempered to existing needs, into the continuing war against disease.

The membership of the committee as now constituted includes the following:

C. P. Rhoads, *chairman;* Florence R. Sabin, secretary; A. R. Dochez, A. Baird Hastings, Charles B. Huggins, Donald F. Jones, C. C. Little, Carl R. Moore, John J. Morton, James B. Murphy, Eugene P. Pendergrass, Howard C. Taylor Jr., M. A. Tuve, M. C. Winternitz.

PHILIP S. OWEN, M.D.

For the Committee on Growth, Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

According to *Science* (Nov. 2, 1945) a sum of $500,000, from funds raised during the cancer campaign in the spring of 1945, is being made available by the American Cancer Society for initial steps in a cancer research program sponsored by this committee in cooperation with the society. Appropriations will be made for chemical, biologic and clinical research and for research in physics. The sum of $50,000 is recommended for fellowships to be given scientific men released from the army who will engage in cancer research.

Awards.—The honorary degree of Doctor of Science has been conferred by the University of Oxford on H. M. Turnbull, professor of morbid anatomy in the University of London and director of the pathological institute of the London Hospital.

The Royal College of Physicians has awarded the Moxon Medal to Sir Alexander Fleming for his discovery of and work on penicillin.

The Theobald Smith Medal of the American Academy of Tropical Medicine has been awarded to Charles M. Wenyon, protozoologist, London.

The United States of America Typhus Commission Medal has been given to Francis G. Blake, Yale University, and Kenneth F. Maxcy, Johns Hopkins University, for "exceptionally meritorious service."

The Weber-Parkes Prize and Medal of the Royal College of Physicians have been awarded to Eugene L. Opie, emeritus professor of pathology of the Cornell University Medical College, for his work on the pathogenesis of pulmonary tuberculosis.

Society News.—The American Association for the Advancement of Science will meet in St. Louis on March 27 to 30, 1946.

The Society of American Bacteriologists will hold its 1946 meeting in Detroit, May 21-24, 1946.

A Request.—The Pasteur Institute, Paris, France, is anxious to procure the following numbers of the ARCHIVES OF PATHOLOGY:

Volume 30, no. 3 (September) 1940; all of the issues in volume 32 (July to December) 1941, and all of the issues for 1942, 1943 and 1944 (volumes 33 to 38 inclusive); also the first 8 issues of 1945 (volume 39, nos. 1 to 6, and volume 40, nos. 1 and 2).

If any reader of the ARCHIVES has any of these numbers that he can spare and will send them to the headquarters office of the American Medical Association, they will be forwarded to the Pasteur Institute.

Books Received

CLASSICAL DESCRIPTIONS OF DISEASE, WITH BIOGRAPHICAL SKETCHES OF THE AUTHORS. By Ralph H. Major, M.D., professor of medicine, University of Kansas School of Medicine. Third edition. Pp. 679, with 158 illustrations. Price $6.50. Springfield, Ill. Charles C Thomas, Publisher, 1945.

This book was first published in 1932. The third edition contains 287 selections from the writings of 195 authors in the field of internal medicine. New selections and a biographic bibliography have been added. There are 158 illustrations, well selected with respect to their historical interest. The subject matter is divided into infectious diseases, lead poisoning, diseases of metabolism, diseases of the circulatory system, diseases of the kidney, respiratory diseases, allergic diseases and diseases of the digestive tract. The selections "deal in the main with clinical medicine." Harvey and Boerhaave are not represented. Harvey's description of a case of rupture of the left ventricle and Boerhaave's account of rupture of the esophagus are historically just as classic as Haller's description (page 406) of a case of fibrocalcareous pericarditis, and succinct biographic sketches of Harvey and of Boerhaave would have enhanced the historical interest of the book. On the jacket it is stated that "the clinical entities, methods of clinical diagnosis, and methods of treatment of disease that are presented cover practically the entire field of medicine and its development from the period of the author of the Papyrus Ebers, who lived a thousand years before Hippocrates, to these modern times." Of course, this is an overstatement of the scope of the book. Several series of selections could be made also from classic descriptions of diseases in surgery, obstetrics, psychiatry, neurology and other specialties. The book does not contain selections from descriptions of cancer, infantile paralysis, industrial or occupational diseases (Ramazzini) other than lead poisoning, Rocky Mountain spotted fever, serum disease, fungous diseases, etc. There is no lack of material for suitable additional sections and selections in new editions to meet the demands created by the contents and the physical quality of the book as it now stands. The book "provides direct and stimulating contact with the founders of nosography."

EXPERIMENTAL CATATONIA: A GENERAL REACTION-FORM OF THE CENTRAL NERVOUS SYSTEM AND ITS IMPLICATIONS FOR HUMAN PATHOLOGY. By Herman Holland De Jong, M.D., associate professor of neuropsychiatry, Duke University Medical School, Durham, N. C. Pp. 225, with 38 illustrations. Price $4. Baltimore: The Williams & Wilkins Company, 1945.

The author and his associates have studied catatonia in ingenious experiments over a period of twenty years. These investigations have shown that experimental catatonia is a form of general reaction of the central nervous system. It can be produced in a great variety of ways: by administration of drugs, by production of anoxemia, by centrifugation and by limiting the blood supply to the liver. The common denominator in all these experiments is obviously a derangement in the metabolism of the central nervous system. The more prolonged states of catatonia were induced by alteration in either the liver or the intestine. This suggests the intriguing theory that abnormal metabolism of the liver results in the development of toxic products, either directly or by the failure of the liver to detoxify certain substances, and that these toxic materials in turn affect the central nervous system and thus induce catatonia. De Jong found that schizophrenic patients with catatonia and also other schizophrenic patients do have altered hepatic function, as shown by the cephalin flocculation test. One should bear in mind, however, as the author emphasizes, that both human and animal catatonia can be elicited in different ways, and therefore all possible causes should be studied. The probability of specific damage of the liver as a cause of catatonic schizophrenia and certain other types of schizophrenia is such a promising lead that it should be followed and studied exhaustively. This book is recommended not only to those interested in psychology but to specialists in internal medicine.

FAMILIAL NONREAGINIC FOOD-ALLERGY. By Arthur F. Coca, M.D., medical director of the Lederle Laboratories. Second edition. Pp. 191. Price $3.75. Springfield, Ill.: Charles C Thomas, Publisher, 1945.

CHRONIC PULMONARY DISEASE IN SOUTH WALES COALMINERS. III. EXPERIMENTAL STUDIES: A. REPORT BY THE COMMITTEE ON INDUSTRIAL PULMONARY DISEASE. B. THE MINERAL CONTENT OF THE LUNGS OF WORKERS FROM THE SOUTH WALES COALFIELD, by E. J. King and G. Nagelschmidt. C. THE ESTIMATION OF COAL AND OF ALUMINUM IN DRIED LUNG, by E. J. King and M. Gilchrist. D. TISSUE REACTIONS PRODUCED EXPERIMENTALLY BY SELECTED DUSTS FROM SOUTH WALES COALMINES, by T. H. Belt and E. J. King. E. THE SOLUBILITY OF DUSTS FROM SOUTH WALES COALMINES, by E. J. King. Medical Research Council Special Report Series no. 250. Pp. 94, illustrated. Price $1.50. London: His Majesty's Stationery Office (New York: British Information Services), 1945.

THE MEDICAL USE OF SULPHONAMIDES. By various authors (edited by F. Hawking and F. H. K. Green). Second edition. Medical Research Council War Memorandum no. 10. Pp. 71. Price 1s 3d. (40 cents). London: His Majesty's Stationery Office (New York: British Information Services), 1945.

CONTRIBUTION TO THE STUDY OF TUBERCULOSIS. By Research Department, National Jewish Hospital at Denver. Volume XVI. 1942-1944. Various pagination. Denver, Colo.: National Jewish Hospital at Denver, 1945.

WHAT PEOPLE ARE. A STUDY OF NORMAL YOUNG MEN. By Clark W. Heath, in collaboration with Lucien Brouha, Lewise W. Gregory, Carl C. Seltzer, Frederic L. Wells and William L. Woods, The Grant Study, Department of Hygiene, Harvard University. Price $2. Pp. 141. Cambridge, Mass.: Harvard University Press, 1945.

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